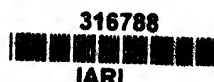




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ERRATA AND AUTHORS' EMENDATIONS

Page 99, Table 1, in heading "arid" should be "acid."

Page 139, lines 4 and 5, insert "do not" between the words "fibers" and "differ."

Page 140, Footnote 5 should be "PETTENKOFFER, M. J. VON. THE RELATION OF THE AIR TO THE CLOTHES WE WEAR, THE HOUSE WE LIVE IN, AND THE SOIL WE DWELL ON. THREE POPULAR LECTURES DELIVERED BEFORE THE ALBERT SOCIETY AT DRESDEN. Abridged and translated by Augustus Hess. 94 p. London. 1873."

Page 142, third line from bottom, "4.12" should be "6 12;" second line from bottom, "10.12" should be "12 12", "20.12" should be "22 12", "40.12" should be "42 12", and "160.12" should be "162.12."

Page 183, line 17, "labrium" should be "labium"

Page 184, Figure 2, fourth line in legend, "190" should be "90."

Page 188, Figure 3, fourth line in legend, "585" should be "385."

Page 189, fifth line from bottom, insert "the drop" between the words "in" and "one"; fourth line from bottom, delete "the drop"

Page 197, fourteenth line from bottom, "assuming" should be "assuring."

Page 199, Figure 1, in legend, the formula should be " $y = -0.0207 + 0.9105x - 3.9490 \log x$,"

Page 200, line 6, " $P=0.01$ " should be " $P<0.01$."

Page 201, line 17, "the second" should be "other."

Page 207, footnote a, "same" should be "serial."

Page 255, fifteenth line from bottom, insert "sandy" between the words "in" and "soil", thirteenth line from bottom, insert "the" between the words "between" and "soil," add "s" to "moisture", and insert "tested" between the words "moistures" and "longevity."

Page 288, lines 9, 24, 28, and 30, "she" should be "he."

Page 367, Table 1, it should be indicated that the species *Microbracon gelechia* (Ashm) was bred from *L. molesta* by the writer.

Page 448, nineteenth line from bottom, "*n. vorax*" should be "*n. ferox*."

Page 450, nineteenth line from bottom, "*brachyurus* De Man (4), 1884" should be "*brachyurus* deMan (4) 1880."

Page 480, footnote 5, page 482, should be on page 480. It refers to "tuber-line" in line 17

Page 483, Table 2, column 1, b should be omitted after "(A)"; column 2, "21.6" should be "41 6."

Page 496, Figure 4, first line in legend, "recovery" should be "production"

Page 497, Figure 5, first line in legend, "recovery" should be "production."

Page 513, sixth line from bottom, "inoculation" should be "isolation."

Page 567, Table 3, forty-seventh scientific name, "*Viola canadensis*" should be "*Viola canadensis*"

Page 578, line 7, "inhabits" should be "inhibits."

Page 691, fourth line from bottom, insert comma after "that" and delete comma after "probably."

Page 774, sixth line from bottom, "the exceptions" should be "four exceptions."

Page 778, line 21, " $r=0.10$ to 0.50 " should be " $r=0.10$ to 0.80 ".

Page 779, line 2, " $r_{SOI\Delta} - r_{SOI\Delta K}$ " should be " $r_{SOI\Delta} - r_{SOI\Delta K}$ "

Page 781, line 13, " $r_{RSOI} > r_{R\Delta}$ " should be " $r_{RCI} > r_{R\Delta}$ ".

Page 781, line 18, delete subscript 4 in " $r_{R\Delta 4}$ ".

Page 821, line 2, "organic" should be "inorganic."

Page 850, line 21, after the word "important" add the following, "since plants take the disease from infected soil and."

Page 874, Table 2, under "Name," "McCulloch" should be "McCulloch."

Page 879, Figure 7, third line of legend, and third line from bottom of page, "McCulloch" should be "McCulloch."

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No. 1

EFFECT OF ENVIRONMENTAL FACTORS UPON THE RESISTANCE OF CABBAGE TO YELLOWS¹

By J. C. WALKER, *Agent, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and Professor of Plant Pathology, University of Wisconsin*, and ROSE SMITH, *Research Associate in Plant Pathology, University of Wisconsin*

INTRODUCTION

In a previous paper (8)² the senior writer described the genetic behavior of resistance in cabbage to the yellows organism, *Fusarium conglutinans* Wr. It was shown that resistance and susceptibility are controlled by a single pair of genes (*Rr*), so far as could be determined from the reaction of the various progenies tested under field conditions in southern Wisconsin on soil naturally infested with the yellows organism.

Several years' work has yielded a number of strains of cabbage which are more constantly and uniformly resistant than any of the mass-selected varieties available to previous workers (2, 4, 8, 9). It was the purpose of the present investigation to study under controlled conditions their reaction to environmental factors, primarily temperature. Such inquiry is important for several reasons. From the practical point of view it is desirable to know to what extremes improved strains will maintain their resistance. The distinction between environmental and hereditary factors in the interpretation of the minor variations in severity of disease in susceptible plants is essential as a guide to further selection. A better understanding of this distinction was also germane to studies of the inherent differences in the relation of the parasite to resistant and susceptible host plants.

Considerable work has been previously done upon the relation of temperature to the development of yellows by Gilman (1), Tisdale (7), and Tims (6). Since these studies have been adequately summarized by Jones and associates (3), it is unnecessary to discuss them in detail here.

METHODS OF EXPERIMENTATION

The naturally infested soil employed in these studies was taken directly from a thoroughly diseased field in southeastern Wisconsin used over a period of several years for testing the resistance of cabbage strains. For artificial inoculation of soil a single-spore isolation of the organism secured from a diseased plant collected in southeastern Wisconsin was grown for several weeks upon a mixture of sand and corn meal. The culture was then pulverized and mixed thoroughly with soil which had been previously sterilized at a pressure of 15 pounds for several hours. The inoculated soil was used in some experiments during the several weeks immediately following its preparation, and it was used again in other trials after storage for several months.

¹Received for publication Aug. 20, 1929; issued June, 1930. This study has been supported jointly by the Department of Plant Pathology, University of Wisconsin, with the aid of a special grant from the general research fund of the university, and by the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

²Reference is made by number (italic) to "Literature cited," p. 15.

Soil-temperature studies were carried out in the Wisconsin soil-temperature tanks described elsewhere (3). Plants were grown in infested soil in galvanized-iron cans which were inserted in tanks held with a reasonable degree of constancy at the desired temperatures. The seedlings were grown in soil free from the yellows organism and transplanted to the cans at the desired time. The cans were then kept at 15° C. or lower until the plants had recovered from transplanting and were then removed to the tanks. In records of these experiments the duration was calculated from the time the cans were placed in the tank, care having been taken to hold the soil temperature previous to that time to a point below 15°, the minimum for disease development. Unless otherwise noted, a common air temperature which fluctuated between 15° and 17° was maintained, except on sunny days, when it rose a few degrees higher.

In the study of air-temperature influences along with soil-temperature effects tanks were used which stood in greenhouses in which the air temperatures were regulated at different levels. Although the temperature of the air was not so constant as that of the soil, it was sufficiently constant to give reasonable contrast between the various levels used.

As indicated in the later discussion of experimental results, the typical appearance of yellows in the leaves was used as the chief criterion in recording the appearance and progress of the disease. In certain cases, however, this was insufficient, because of variation in symptoms under different environments. This was especially true at the higher soil temperatures in the case of resistant plants, where stunting of the plants and unnatural mottling of the leaves, not typical symptoms of the disease, were common as a result of exposure to the extreme environment. In these investigations the influence of soil moisture was not studied. For each series, however, the soil moisture was kept uniform throughout by frequent weighings and the addition of sufficient water to maintain the original weight of all pots. Following the evidence from Tisdale's work (7) that moderately dry soil gave the greatest quantity of disease, the moisture content was kept at about 60 per cent of the water-holding capacity.

SOURCE OF HOST MATERIALS

As has already been explained in a previous paper (8), most commercial varieties of cabbage are extremely susceptible to yellows. In each variety, however, there are ordinarily a few plants that are resistant under field conditions. These are in practically all cases heterozygotes so far as resistance is concerned, and, resistance being dominant, they withstand the attack of the yellows organism. By pure-line selection from such individuals, lines were secured which are homozygous for resistance under field conditions, in Wisconsin at least and where tested in other localities. In like manner individuals selected from commercial varieties growing on noninfested soil in most instances yield progenies that are practically 100 per cent susceptible. Such lines are herein regarded as homozygous for the susceptible character.

A third type of material used in this connection is that referred to as mass-selected resistant. Examples of this are Wisconsin All Seasons and Wisconsin Hollander, resistant varieties now in general commercial use. These differ from the homozygous-resistant lines

in that they were derived from commercial types, not by pure-line selection but by mass selection from the surviving plants on infested soil. They have thus remained to some degree heterozygous for resistance and in each generation continue to produce some purely susceptible individuals. As was pointed out earlier, resistant strains of this type were the only ones available to Tisdale (7) and to Tims (6). For purposes of more direct comparison the same varieties they used, Wisconsin Hollander and Wisconsin All Seasons, were included in these studies.

Thus the following two resistant and two susceptible types of material were used:

(1) The homozygous-resistant lines were progenies of individual plants, or mixtures of such progenies, which were proved to be homozygous by progeny tests in the field. Progenies 40-350s, 40-353s, 40-354s, 40-420s, 40-421s, 40-426s, 40-524s, 40-530s, and 40-535s were secured by self-pollination of individual plants selected from the All Head Early variety. 40-26-F was a mixture of remnants of selfed progenies from the same variety. 30-25-A was from the seed of several homozygous-resistant plants selected from the Copenhagen Market variety and allowed to be cross-pollinated by insects in an isolated location. 20-27-C was a mixture of remnants from selfed progenies of plants selected from the Jersey Wakefield variety. 20-29-A was the second generation from a homozygous resistant individual of the same variety.

(2) The mass-selected resistant varieties were secured from a reliable commercial source of these seeds. Wisconsin Hollander, Wisconsin All Seasons, and All Head Select were used.

(3) Homozygous-susceptible lines were from plants proved by progeny tests in the field to be homozygous for susceptibility. HG-5s was a selfed progeny from an F_1 hybrid plant resulting from a cross between an All Head Early and a Glory of Enkhuizen susceptible individual. HG-27-A was a mixture of sister progenies coming from the same original cross as HG-5s. Reciprocal crosses were made of two F_1 hybrid plants coming from a cross between an All Head Early and a Copenhagen Market susceptible individual; the flowers of these two plants (HC-19 and HC-3) were not emasculated, but a single camel's-hair brush was worked over the blossoms of each plant and the seed of each was saved separately. Seed from plant HC-3 was used and is designated as HC-3 \times 19B. C-29-A was a homozygous-susceptible line from the Copenhagen Market Variety.

(4) Two commercial susceptible varieties, Copenhagen Market and Danish Ballhead, were used. Although these were not quite so homogeneous as the homozygous-susceptible lines, they were shown by field tests to contain a very small percentage of resistant individuals.

In addition to the above-mentioned lots, certain F_1 and F_2 progenies from crosses between resistant and susceptible lines were studied. These are described later in the text.

EXPERIMENTAL RESULTS

COMPARATIVE STUDY OF RESISTANT AND SUSCEPTIBLE LINES

INFLUENCE OF SOIL TEMPERATURE

The plants used in experiment 1 had grown in noninfested soil for about six weeks before they were transplanted to infested soil. Two homozygous-resistant lines (40-535s and 30-25-A) were compared.

with one commercial susceptible variety (Copenhagen Market). Table 1 shows that the severity of the disease in the susceptible variety increased with rise in soil temperature, reaching a maximum at 26° to 30° C. This is in accord with the findings of Tisdale (7) and Tims (6) for other susceptible varieties. In the case of the resistant strains, however, there was complete absence of disease at 20° and 22°. At 24° no external symptoms appeared, but upon careful examination of the vascular system there were found a few brownish streaks not unlike those present in greater abundance in susceptible plants at this and other temperatures. All attempts to isolate the fungus from such stems at 24° and 26° were unsuccessful. The significance of the browning of the veins in resistant lines at this temperature will be discussed later. In one resistant line (30-25-A) a few plants showed external evidence of disease at 28°, and both strains had a few such cases at 30°. It thus appeared that the homozygous-resistant lines were exhibiting uniform resistance in this case at a constant soil temperature as high as 26°. This was quite different from the results secured by Tisdale (7) and Tims (6) with the mass-selected resistant varieties, which showed considerable disease at 20° and comparatively little resistance above that point.

TABLE 1.—*Development of yellows in a commercial susceptible cabbage variety and two homozygous-resistant progenies when grown on naturally infested soil at various constant soil temperatures*

Soil temperature (°C.)	Occurrence of yellows in—					
	A susceptible variety ^a		A resistant variety ^b		A resistant variety ^c	
	Plants	Diseased	Plants	Diseased	Plants	Diseased
	Number	Per cent	Number	Per cent	Number	Per cent
20	10	60	20	0	23	0
22	10	70	24	0	22	0
24	9	89	24	0	23	0
26	9	100	24	0	23	0
28	8	100	24	0	23	17
30	9	100	24	25	23	13

^a Copenhagen Market.

^b Selected from All Head Early, 40-535s.

^c Selected from Copenhagen Market, 30 25 A.

TABLE 2.—*Development of yellows in two homozygous-susceptible cabbage progenies and eight homozygous-resistant progenies when grown on naturally infested soil at various constant soil temperatures*

Description of strain	Strain No.	Occurrence of yellows at soil temperature of—					
		24° C.		26° C.		28° C.	
		Plants	Diseased	Plants	Diseased	Plants	Diseased
		Number	Per cent	Number	Per cent	Number	Per cent
Homozygous-susceptible	HG-5s	10	100	10	100	10	100
	HC-3×19B	7	100	7	100	6	100
	40-350s	7	0	7	0	7	57
	40-353s	10	0	9	11	10	0
Homozygous-resistant. From All Head Early	40-354s	8	0	8	0	8	25
	40-420s	9	0	9	0	9	22
	40-421s	10	0	9	0	9	22
	40-426s	10	0	10	0	10	10
	40-524s	7	0	6	0	7	0
	20-27-C	7	0	10	20	10	40

In experiment 2 (Table 2) the study was extended to two homozygous-susceptible lines (HG-5s, HC-3 \times 19B), a number of homozygous-resistant progenies selected from All Head Early (40-350s, etc.), and a mixture of several homozygous-resistant progenies from Jersey Wakefield (20-27-C). In this instance the plants were much younger when exposed to infection, which exposure took place only 26 days from the time the seed was sown. The experiment was continued for 40 days. Only the critical temperatures 24°, 26°, and 28° C. were used. At all temperatures the susceptible strains succumbed completely. At 24° all the resistant lines remained free from disease symptoms. At 26° two progenies showed slight disease, and at 28° all but two progenies showed more or less disease. Thus in comparison with experiment 1, these lots were as resistant at 24°, but two of them were less stable at 26°, and most of them less so at 28°.

In experiment 3 a homozygous-susceptible line (HG-5s), two homozygous-resistant lines (40-420s and 20-27-C), and three mass-selected resistant varieties were used. (Table 3.) The plants in this case were 32 days old when exposed to the various soil temperatures (18°-33° C.), and the experiment was continued for 33 days. As noted earlier by Tisdale (7) and Tims (6), the disease developed very slowly at 18°, even in the susceptible strain, while its incidence also was much reduced at 33°. These extremes appear to limit the progress of the parasite materially, and this probably accounts in a large measure for the reduction in the amount of the disease. The behavior of the homozygous-susceptible and the homozygous-resistant lines otherwise coincides in general with that observed in experiments 1 and 2. As noted in the experiments of Tisdale and Tims, a small percentage of plants of Wisconsin Hollander and Wisconsin All Seasons became diseased at 21°, and the percentage increased with the rise in temperature up to 30°. The difference between the homozygous-resistant lines and mass-selected varieties appears at 21° and higher temperatures. It is evident that the latter, though quite resistant under Wisconsin field conditions, are inherently much more susceptible than the homozygous lines, while their exposure to constant temperatures above 21° reveals that they are unsuited to such conditions. The cans from this series were photographed near the close of the experiment, and some of them are shown in Figure 1.

TABLE 3.—*Development of yellows in susceptible and resistant cabbage progenies when grown on naturally infested soil at various constant soil temperatures*

[Ten plants of each strain at each temperature except 33°, where there were 20 plants]

Description of strain or variety	Strain No. or variety	Percentage of plants yellowed when grown at soil temperature of—					
		18° C.	21° C.	24° C.	27° C.	30° C.	33° C.
Homozygous-susceptible..	HG-5s	50	100	100	100	100	100
Homozygous-resistant..	40-420s	0	0	0	0	40	25
	20-27-C	0	0	0	10	80	35
Mass-selected resistant..	Wisconsin Hollander	20	30	50	80	90	25
	Wisconsin All Seasons	0	20	50	20	70	50
	All Head Select	0	20	20	60	80	60

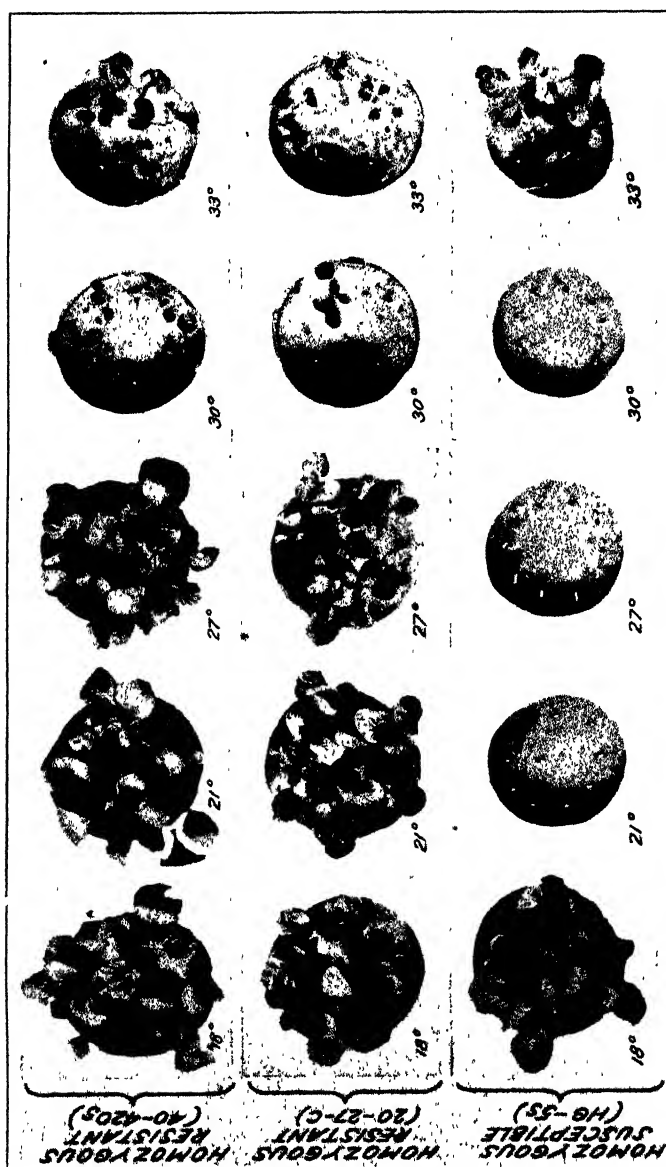


FIGURE 1.—Pots from experiment 3 (Table 3), photographed near the close of the experiment. The white markers indicate where the dead plants were removed. Homozygous resistant progenies (40-4205 and 20-27-C) and a homozygous susceptible progeny (H9-55) are shown. All plants of 40-4205 remained healthy from 18° to 27° C., whereas at 30° the plants were started to wilt and died. They did not show typical yellowing, however. The same is true of 20-27-C, except that one plant died at 21°. Some plants of the susceptible line (H9-55) were still healthy at 18° but at 21° and up to 30° all were diseased and all but one had died. At 33°, as in the resistant lines, the disease was less destructive than at 30°. Apparently the activity of the fungus was reduced at this temperature.

In the three experiments just described certain new facts are brought out. In the first place, the homozygous-resistant strains, so called because of their perfect resistance under repeated field tests, are extremely resistant at quite high temperatures. Plants of the ages used were practically all stable up to and including constant temperatures of 26° to 27° C. Above this point the plants became stunted, showed a pale yellowing of the leaf parenchyma, and exhibited some browning of the vascular bundles. The external appearances of disease were not typical of those in the susceptible lines at these or lower temperatures. The browning of veins was very rarely accompanied by the presence of the organism.

It was important to determine whether this peculiar reaction of resistant plants at high temperature was due to the attack of the parasite or to the direct effect of the temperature upon the plants. The behavior of plants in infested and in noninfested soil over a range of temperature was therefore studied. (Experiment 4.) In order to make the infested and the noninfested soil as comparable as possible, a quantity of the latter was divided and to one portion inoculum of *Fusarium conglutinans* was added. A series of soil-temperature tanks ranging at 2-degree intervals from 18° to 28° C. were then set up so that in each tank there was one can each of homozygous susceptible plants (C-29-A) and one can each of homozygous resistant plants (20-29-A) in inoculated and in uninoculated soil. The plants were 43 days old at the beginning of the experiment, and the latter was run for 30 days. Six plants were placed in each can. In the inoculated soil the results were similar to those in the naturally infested soil in experiments 1, 2, and 3. The susceptible plants showed typical yellows, the severity increasing with the temperature. The resistant plants remained perfectly healthy up to 24°; they showed some atypical signs at 26°, and the atypical symptoms were severe at 28°. In the uninoculated soil no signs of disease whatever were noted, even at the highest temperature. This fact shows that this rather abrupt appearance of pathological symptoms in the resistant plants at 26° and above is the result of the yellows organism in the soil and not the effect of high temperature alone. The cans from the 24° and the 28° tanks, photographed on the thirteenth day, are shown in Figure 2.

Histological studies subsequently reported (5) indicate that there is invasion of the root tips of homozygous-resistant plants with little or no penetration of the vascular system. Furthermore, at 28° and above, the root systems of cabbage are at their low ebb of growth, while the causal organism is most active (7). The reduced rate of root growth, with invasion of the absorbing regions of the root system, is probably responsible for the gradual stunting and death of the resistant plants at 28° and above. Moreover, it is to be emphasized that this breaking down of resistance is of quite a different order in external appearance and in internal histology from the normal development of disease in susceptible plants at these or other temperatures.

The distinct difference between commercially successful mass-selected resistant varieties and pure-lined homozygous-resistant lines in their temperature reactions is the second matter of importance to be noted in these results. Many plants of the mass-selected strains behaved like susceptible ones under these controlled conditions, which is in accord with Tisdale's (7) and Tims's (6) results. While this may

not appear to be in accord with the fact that these varieties are commercially successful upon infested soil, it should be pointed out that it is not uncommon to find a field of these mass-selected varieties with as high as 50 per cent of the plants showing slight symptoms of disease. As has been suggested before (8), it is possible that in the selection of these varieties mildly susceptible individuals were not eliminated and as a result they are apparently present in considerable numbers. Under average seasonal conditions where the crop is started during the cooler spring months so that the plants reach considerable size before infection occurs, these mildly susceptible individuals are not easily distinguished. The test under controlled environment is ap-

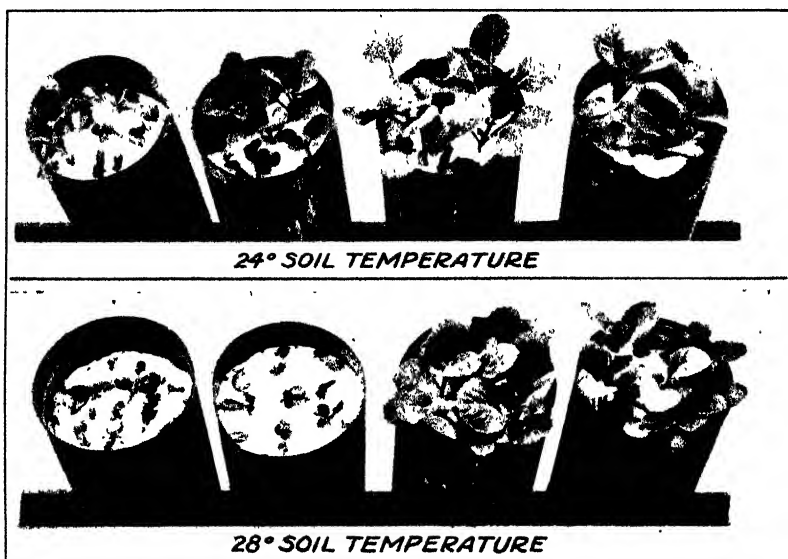


FIGURE 2.--Comparison of resistant and susceptible lines of cabbage upon inoculated and uninoculated soil at 24° and 28° C. Cans in left-to-right order in each row contain (1) susceptible plants in inoculated soil; (2) resistant plants in inoculated soil, (3) susceptible plants in uninoculated soil; (4) resistant plants in uninoculated soil. See further explanation in the text

parently more severe and the differences between them and homozygous-resistant plants are accentuated.

INOCULATED STERILIZED SOIL

In the first three experiments naturally infested soil was used. While this method yielded results more comparable with those secured under field conditions, it did not afford a picture of the behavior of the plants exposed to the yellows organism when it was removed from competition with other soil organisms and thus allowed to accumulate in greater abundance. In experiment 4 the plants were 50 days old when exposed to various soil temperatures. A commercial susceptible variety, a mass-selected resistant variety, and a homozygous-resistant strain were used. There were four to seven plants in each can. The percentages of diseased and of dead plants were recorded after 30 days. The final results are shown in Table 4.

TABLE 4.— *Development of yellows in a susceptible cabbage variety, a mass-selected resistant variety, and a homozygous-resistant progeny when grown in naturally infested and inoculated sterilized soils at various constant soil temperatures*

[In each individual lot 7 plants were used, except of Wisconsin Hollander at 26°, where only 6 plants were used in naturally infested soil, and at 28°, where 5 and 6 plants were used, respectively, in naturally infested and inoculated sterilized soil]

Soil temperature (° C.)	Occurrence of yellows in susceptible commercial (Copenhagen Market) variety grown in —				Occurrence of yellows in mass-selected resistant (Wisconsin Hollander) variety grown in —				Occurrence of yellows in homozygous-resistant (40-5308, selected from All Head Early) vari- ety grown in —			
	Naturally infested soil		Inoculated sterilized soil		Naturally infested soil		Inoculated sterilized soil		Naturally infested soil		Inoculated sterilized soil	
	Dis- eased plants		Dis- eased plants		Dis- eased plants		Dis- eased plants		Dis- eased plants		Dis- eased plants	
	P	ct	P	ct	P	ct	P	ct	P	ct	P	ct
20	86	57	100	100	14	0	43	14	0	0	0	0
22	100	86	100	100	57	14	43	29	0	0	0	0
24	100	100	100	100	100	57	100	86	0	0	0	0
26	100	100	100	100	100	33	100	71	0	0	100	0
28	100	100	100	100	100	80	100	100	* 100	* 14	* 100	* 43
30	100	100	100	100	100	100	100	100	* 100	* 100	* 100	* 100

* These plants did not show typical symptoms of yellows but were nevertheless stunted and showed yellowish spots on the leaves, dying back of the leaf margins, and more or less browning of the veins. Practically all attempts to isolate the organism from them were unsuccessful. Differences are illustrated in Figure 3.

It is clear that the increased potentiality of the inoculum which results from sterilization of the soil to remove the soil flora, temporarily at least, from competition with the yellows organism has an immediate effect upon the disease. The incubation period is somewhat shortened and the progress of the disease is hastened. Aside from these effects it is quite consistently noticeable that the total percentage of plants diseased in the susceptible and mass-selected resistant groups is increased in the inoculated soil. This is probably the result, in part at least, of a more uniform distribution of the parasite in the soil. The relative differences between susceptible and resistant strains are not changed; but the homozygous-resistant line showed, in inoculated sterilized soil, the pathological response characteristic of this type at high temperatures at a slightly lower point than in previous experiments wherein naturally infested soil was used.

INFLUENCE OF AIR TEMPERATURE

In the first five experiments the air temperature during most of the time was at 15° to 17° C., although for a few hours during sunny days it rose a few degrees higher. This is somewhat lower than might often obtain under natural conditions during midsummer. Consideration was next given to the influence of the air temperature as well as of the soil temperature upon the expression of resistance.

In experiment 6 a homozygous-susceptible line (HG-27-A) and a homozygous-resistant line (40-26-F) were included. Plants 45 days old of each were used, and the experiment was run for 27 days in naturally infested soil. Soil temperatures of 20° and 24° were maintained at air temperatures of 15° to 17° and of 28°, respectively. In the susceptible variety the disease developed most rapidly at the 28°

air temperature in the two respective soil temperatures, which is in accord with the earlier findings of Tims (6). The final results (Table 5) show, however, that even at this air temperature the homozygous-resistant line remained healthy.

TABLE 5.—*Development of yellows in susceptible and resistant lines of cabbage at various air and soil temperatures*

Temperature of—		Occurrence of yellows in—			
Air	Soil	Susceptible line		Resistant line	
		Plants	Diseased	Plants	Diseased
° C.	° C.	Number	Per cent	Number	Per cent
15	20	5	100	10	0
	24	5	100	10	0
28	20	5	100	10	0
	24	5	100	10	0

EFFECT OF WOUNDING THE ROOT ON INFECTION AND RESISTANCE

It became of interest to determine whether or not severe pruning of the root system would permit the development of disease in resistant plants. Plants 10 weeks old of a homozygous-resistant strain (40-535s) and a commercial susceptible variety (Copenhagen Market) were transferred to a bench of naturally infested soil, a portion of each strain being handled as follows: (1) Transplanted with the least possible injury to the roots; (2) roots pruned moderately before resetting; and (3) all lateral roots pruned close to the taproot. The disease was recorded for each lot as it appeared, and the data are summarized in Table 6. It is significant that even the severest degree of root pruning did not bring about infection of the resistant strain. In the susceptible strain the appearance of the disease in slightly pruned plants was delayed several days as compared with the moderately and severely pruned plants, but at the end of the period the total infections in the three lots were not significantly different.

TABLE 6.—*Effect of varying degrees of root pruning at transplanting upon the occurrence of yellows in a resistant and a susceptible strain of cabbage*

Strain or variety	Degree of root pruning	Total number of plants	Number of yellowed plants at end of—							
			17th day	19th day	23d day	25th day	30th day	33d day	37th day	44th day
40-535s (resistant)	Slight	19	0	0	0	0	0	0	0	0
	Moderate	20	0	0	0	0	0	0	0	0
	Severe	20	0	0	0	0	0	0	0	0
Copenhagen Market (susceptible)	Slight	10	0	0	4	4	7	9	9	9
	Moderate	16	3	4	6	7	7	13	13	13
	Severe	17	2	4	4	5	7	12	14	15

GROWING CONDITIONS BEFORE EXPOSURE TO THE PARASITE IN RELATION TO SUSCEPTIBILITY AND RESISTANCE

Throughout the experiments just reported plants for transplanting were commonly grown at comparatively low temperatures—about 15° C.—and were then transferred to infested soil and placed at various

soil and air temperatures. The question arose as to whether the conditions under which a plant is grown before exposure to the parasite under a given environment have any important bearing upon susceptibility or resistance. In fact Tims (6) suggested that plants grown at a comparatively high temperature became diseased less readily than those grown at lower temperatures more favorable for growth of the host. An experiment was therefore planned to yield some evidence on this point.

Seeds of a homozygous-resistant line (20-27-C) and of a homozygous-susceptible line (HC-27-A) were sown in cans of noninfested soil. They were placed in one set of soil-temperature tanks running at 20°, 24°, 28°, and 32°, at each of two air temperatures, 15°-17° and 28°. Thus each of these two varieties was growing under eight different combinations of air and soil temperature. After 32 days they were transplanted to naturally infested soil. After standing 3 days at about 20° for the plants to recover from transplanting, the cans were so divided that one half of the plants from each combination of temperatures was kept at a soil temperature of 24° and an air temperature of 28°, while the other half was held at 24° soil and 15°-17° air. The plants were thus held for 23 days. By that time every plant in the susceptible line was diseased and most of them were dead. No disease appeared in any of the resistant lines throughout the series. The rate of appearance of the disease and death of the susceptible plants, as shown in Table 7, does not indicate any marked predisposing influence of the growing conditions previous to infection upon disease development in plants of this age. It appears that the method employed of producing the plants at temperatures favorable to growth and later shifting them rather suddenly to various temperatures for exposure to the parasite does not introduce a significant experimental error.

TABLE 7.- *Relative development of yellows in susceptible cabbage plants^a grown for about five weeks in noninfested soil at various soil and air temperatures and then transplanted to infested soil and exposed to a soil temperature of 24° and air temperatures of 15°-17° and 28° C.*

Air temperature after transplanting (°C)	Temperatures before transplanting from clean soil		Total number of plants	Number of yellowed plants at the end of—				Number of dead plants at the end of—						
	Air	Soil		10 days	12 days	14 days	16 days	12 days	14 days	16 days	18 days	19 days	22 days	23 days
15-17.	15-17	20	5	2	4	5	—	0	0	0	0	1	3	—
		24	5	0	4	5	—	0	0	0	0	3	5	—
		28	5	4	4	5	—	0	2	3	4	4	5	—
		32	5	1	4	5	—	0	0	2	2	2	4	—
		20	5	0	5	5	—	0	0	0	3	3	5	—
		24	5	0	0	1	5	0	0	0	0	0	3	—
	28	28	5	0	1	3	5	0	0	1	1	3	4	—
		32	4	2	3	4	—	0	1	1	1	3	4	—
		20	5	4	4	5	—	0	0	2	3	3	4	—
		24	5	0	5	5	—	0	0	3	4	4	4	—
		28	4	0	3	3	4	1	1	3	4	—	—	—
		32	4	0	2	3	4	0	0	1	2	3	3	—
28	28	20	5	2	4	4	5	0	3	3	4	4	5	—
		24	5	0	3	3	5	0	3	3	3	3	4	—
		28	4	3	3	4	—	0	2	2	4	—	—	—
		32	4	1	2	4	—	0	1	1	4	—	—	—
		20	5	—	—	—	—	—	—	—	—	—	—	—
		24	5	—	—	—	—	—	—	—	—	—	—	—

^a HC-27-A.

STUDY OF REACTION OF RESISTANT-SUSCEPTIBLE HYBRIDS

In the light of the evidence brought together in the foregoing pages, it is of interest to consider the reaction of resistant-susceptible hybrids as reported earlier (8).

F₁ HYBRIDS

The F₁ hybrids from resistant and susceptible parents were first studied. Several progenies were grown in both inoculated sterilized and naturally infested soil at various temperatures. The hybrids, which were fully resistant in field tests, were found to be quite as resistant as homozygous-resistant plants except for the fact that in the case of four progenies they showed stunting and high-temperature symptoms at slightly lower temperatures. (Table 8.) In a fifth case (20-27-D) no pathological effects were noted at 26° C. in naturally infested soil. Certain of the lots are shown in Figure 3.

TABLE 8.—Development of yellows in F₁ cabbage hybrids from resistant-susceptible crosses, and commercial susceptible and homozygous-resistant lines

Age of plant	Duration of experiment	Description of strain or variety	Strain No. or variety	Occurrence of yellows in plants grown on—							
				Naturally infested soil at—				Inoculated sterilized soil at—			
				24° C.		26° C.		24° C.		22° C.	
				Plants	Diseased	Plants	Diseased	Plants	Diseased	Plants	Diseased
Days	Days			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
50	30	Susceptible	Copenhagen Market	7	100	7	100	7	100	7	100
		Resistant	40-5308	7	0	7	0	7	0	7	100
		F ₁ hybrids	11-2×5-21	7	0	4	100	4	100	4	100
		Susceptible	Copenhagen Market	4	100	4	100	4	100	4	100
82	28	Resistant	40-5308	4	0	4	0	4	0	4	100
		F ₁ hybrids	5-32×C-1	4	0	4	100	4	100	4	100
		do	40-335×11-35	4	0	4	100	4	75	4	100
		do	5-32×11-35	4	0	4	100	4	100	4	100
40	32	do	20-27-D	10	0	10	0				

* The symptoms in these cases were not those of typical yellows that occurred in the susceptible strain but were the atypical signs current in resistant strains at high soil temperature as described elsewhere in the text.

F₂ HYBRIDS

From the evidence already presented it was to be expected that F₂ hybrid progenies would react according to the constitution of their segregants. Approximately 25 per cent, being homozygous-resistant, should survive as transplanted seedlings up to 26° C. Approximately 50 per cent, being heterozygotes, should survive at 24° and some become stunted at higher temperatures in naturally infested soil. The remaining one-fourth, being homozygous-susceptible, should succumb at 22° or above. Variation in the progress of the disease in the susceptible plants should also result, depending upon soil temperature.

The experiment reported in detail was conducted with a single F₂ progeny (5H1A) which in field trials showed 28.6 per cent diseased plants in 1926 and 22.2 per cent in 1927. Plants 37 days old were exposed to a range of soil temperatures in naturally infested soil. At

18° and 20° somewhat fewer than the expected number were affected; at 22° exactly one-fourth were diseased. (Table 9.) At 24° slightly above 25 per cent were diseased. At 26° the percentage was significantly higher, while at 28° only about one-fourth survived. At

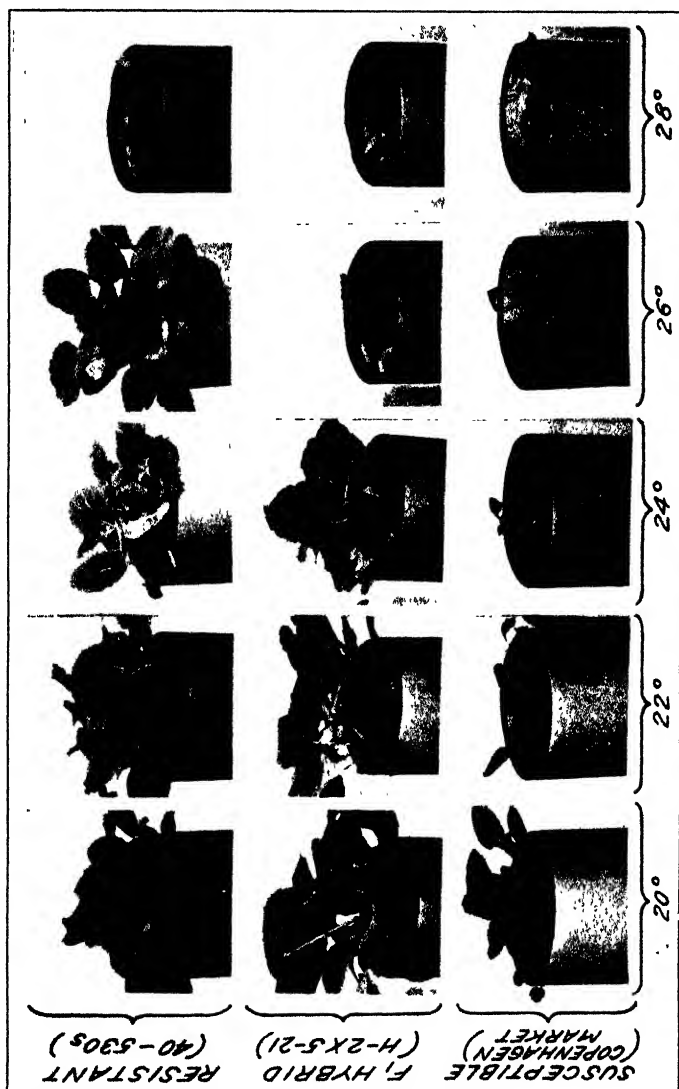


FIGURE 3.—Comparison of development of yellows in a homozygous-resistant progeny (40-530s), a susceptible variety (Copenhagen Market), and a hybrid progeny from a cross between a resistant and a susceptible plant (H-2X5-21). Plants grown on infested soil at various soil temperatures. (For details see Table 9.) The homozygous-resistant strain was healthy at 26° C. but decidedly stunted at 28°, whereas the hybrid plants were healthy at 24° but stunted at 26°. One plant of the susceptible strain was still healthy at 20°, but all plants were diseased or dead at 22° and above.

26° part of the plants and at 28° all of those which survived showed distinct stunting of the type observed in heterozygous and homozygous-resistant plants at these temperatures. No attempt was made to differentiate the two types when the counts were recorded.

TABLE 9.—*Development of yellows in an F₂ hybrid progeny from a cross between a homozygous-susceptible and a homozygous-resistant parent*

Soil temperature °C.	Total number of plants	Yellowed plants	
		Number	Per cent
18	58	12	20.6
20	59	12	20.3
22	60	15	25.0
24	58	16	27.5
26	60	19	31.6
28	60	44	73.3

SUMMARY

The purpose of the study reported in this paper was to determine the effect of environmental factors upon the resistance or susceptibility in various strains of cabbage to yellows (*Fusarium conglutinans*).

The hosts tested consisted of homozygous-resistant progenies, homozygous-susceptible progenies, mass-selected resistant varieties in commercial use, and commercial susceptible varieties.

In the susceptible strains and mass-selected resistant varieties the typical disease symptoms appeared in increasing percentages with increase in soil temperature to about 28° C., and were retarded somewhat at 33°. In homozygous-resistant strains no evidence of disease was found up to 24° in naturally infested soil when young transplants were used. Slight evidence of disease was found in a few cases at 26° and to a greater degree at still higher temperatures. At this higher range the symptoms were not typical of yellows, and the fungus was rarely isolated from such plants. The homozygous-resistant lines, therefore, reacted in a distinctly different manner from the susceptible and mass-selected resistant types.

The increase of inoculum secured by steam sterilization of the soil and reinoculation with a pure culture of the yellows organism resulted in more uniform infection and more rapid disease development in susceptible and mass-selected resistant types, but had no effect upon the homozygous-resistant lines, except that the atypical high-temperature symptoms were evident at a slightly lower temperature.

Increase in air temperature up to 28° C. hastened the development of disease but did not alter the distinct difference in reaction between homozygous-resistant lines and the other types used.

Severe pruning of the root system during the process of transplanting served to shorten the incubation period in susceptible plants, but it did not facilitate the production of yellows in homozygous-resistant plants.

The temperature at which plants were grown prior to exposure to the parasite did not appear to affect the rate of disease development, nor did it have any influence upon the stability of resistance in homozygous lines.

Heterozygous plants, i. e., those resulting from crosses between resistant and susceptible parents, reacted as homozygous resistant except that they usually showed atypical high-temperature symptoms at a slightly lower temperature.

At soil temperatures around 22°–24° C. transplants of F₂ segregating progenies from resistant-susceptible crosses showed approxi-

mately 25 per cent typical susceptible plants, as they had previously shown in the field. As the temperature increased above this point the atypical high-temperature symptoms appeared, as was expected, in the heterozygous and homozygous resistant members of the population. As the temperature fell below this point the full expression of typical disease symptoms in homozygous-susceptible plants decreased

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A CYTOLOGICAL STUDY OF CABBAGE PLANTS IN STRAINS SUSCEPTIBLE OR RESISTANT TO YELLOWS¹

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INTRODUCTION

Yellows of cabbage (*Fusarium conglutinans* Wr.) and the progress in its control through the development of resistant varieties have been discussed elsewhere (7, 8, 9, 16).³ Out of this breeding work certain pure lines of cabbages have been secured (14), some of which are completely resistant and others completely susceptible under field conditions. In genetic studies with this material resistance has been shown to behave as a single dominant Mendelian factor.

When dependable homozygous-resistant and susceptible lines became available the writers began a study to determine if possible the cause of this marked resistance to the yellows organism possessed by certain lines of cabbage and not by others. The problem was approached from two angles: (1) The influence of a number of environmental factors upon the stability of resistance was studied. The results are presented in the paper immediately preceding this one (15). (2) A cytological study of infection and the relation of host to parasite in resistant as compared with susceptible lines was undertaken. The purpose of this study was to find the answers to several questions. Does the yellows organism invade the roots of resistant plants? If so, how extensively are the roots attacked? Are there any visible morphological differences between resistant and susceptible roots? Are there any visible differences in the reaction of the two types of host to the invading parasite?

MATERIALS AND METHODS

HOST MATERIALS

As pointed out in a previous paper (14), most of the commercial varieties of cabbage, with the exception of those recently developed for resistance to yellows, contain a very large percentage of susceptible plants and practically always a small percentage of resistant ones. From such commercial strains were made selections which appeared to be homozygous for susceptibility under field conditions. In the course of this cytological work both commercial and selected materials were used. During the early part of the investigation, particularly when selected susceptible material was not yet available, a

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² The writers wish to acknowledge with thanks the advice and suggestions of Miss Helen Johann and Prof. L. R. Jones during the progress of this investigation.

³ Reference is made by number, (italic) to "Literature cited," p. 34.

strain of the Copenhagen Market variety listed as Vaughan's Reselected Copenhagen, and designated in this paper as VRC, was used. In tests both in the field and in the greenhouse under controlled environmental conditions this strain was found to be very susceptible. Later, seed was used from a cross (C-16 \times 31B) between two Copenhagen Market plants, a progeny which had been shown to be homozygous for susceptibility by the fact that it succumbed very promptly to the disease under field and greenhouse conditions. A third lot, equal to the latter in susceptibility, was a mixture of seeds from tested homozygous-susceptible plants coming from a cross between the two varieties All Head Early and Glory of Enkhuizen. This is referred to as HG-27A.

For the study of the resistant host several progenies from all Head Early were used. These were secured by self-pollination of individual plants from a line that had been selected for several years. The progenies used in this work were tested on infected soil in the field and in the greenhouse and showed no signs of disease except for certain abnormal symptoms when grown at a constant soil temperature of 26° C. or above. The progeny numbers were 40-350s, 40-353s, 40-354s, 40-420s, 40-421s, 40-426s, and 40-524s. The behavior of these and other progenies under varying conditions is discussed in another paper (15, Table 2). They represent what has been referred to as "homozygous-resistant" plants so far as resistance to the yellows organism is concerned (14).

SOURCE OF INOCULUM

The culture of *Fusarium conglutinans* used was isolated in November, 1926, from diseased cabbage grown at Racine, Wis. From a single-spore culture the inoculum was increased on potato-dextrose agar. Isolations of the fungus from Fayetteville, Ark., Crystal Springs, Miss., and Racine, Wis., respectively, were also employed for comparative purposes.⁴

METHODS OF STUDYING RELATION OF FUNGUS TO HOST

Fungal penetration was studied in young seedlings grown on agar. The seeds were surface sterilized by immersing them in a 1-1,000 solution of corrosive sublimate for five minutes. After being thoroughly washed in several changes of sterile distilled water they were placed in Petri dishes of soil-extract agar. Twenty-four to forty-eight hours later the uncontaminated seedlings were transferred to other Petri dishes of soil-extract agar and arranged in a row, about equidistant from one another, with the roots all pointing in the same direction. Inoculum was applied at definite points—in front of the rootcap, along the sides of the root, and in the vicinity of root hairs. The Petri dishes, arranged in wire baskets so that the seedlings were held in a normal position, were placed in a constant-temperature chamber kept at 24° C.

Studies of the distribution of the fungus in the host were made with older plants grown in soil. Surface-sterilized seeds were sown in a cool greenhouse in flats of sterilized soil. When 30 to 45 days old the seedlings were transplanted to cans of artificially inoculated

⁴ These last 3 cultures were provided by L. M. Blank.

soil. This was prepared by autoclaving field soil at a pressure of 10 pounds for three hours on two alternate days. Twenty-four hours later the soil was inoculated uniformly with the fungus, which had been grown in pure culture on a mixture of corn meal and sand. After transplantation of the seedlings the cans were placed in tanks in which the desired constant soil temperatures were maintained.

Considerable difficulty was experienced in removing young growing roots from the soil with their parts intact. The best results were secured by taking up a plant with a large clump of soil and gently agitating it in a vessel of water until it was freed of soil particles. After being surface sterilized, thin slices of the sections being fixed were plated out on potato-dextrose agar. Those sections which yielded *Fusarium conglutinans* and no other fungus were dehydrated and infiltrated with paraffin.

Medium chrom acetic, Gilson's, and formol acetic alcohol fixatives were employed. For detailed study of the fungus in the host Gilson's fixative gave the most satisfactory results. The sections were cut from 10μ to 13μ in thickness. Flemming's triple and Delafield's haematoxylin and safranin were the stains used.

ANATOMY OF THE NORMAL ROOT

For the sake of clarity in the account that follows, a brief description of the anatomy of the young healthy cabbage root is given. As no morphological differences were observed in the resistant and susceptible strains studied, they are not discussed separately.

The growing tip of the young root is protected by a thin cap of cells. Slightly back of it the outermost layer of the primary meristem, the forerunner of the epidermis, differentiates. Its cells are radially elongated and rich in protoplasm. Tangential division of its cells near the apex renews the rootcap from within as its outermost parts are lost. (Fig. 1.) Posteriorly, the cells become elongated axially and the outside wall may be pushed outward to form root hairs.

The primary cortex is made up of four or five rows of elongated cylindrical cells separated from one another by large intercellular spaces. The layer surrounding the endodermis shows peculiar thickenings within 2 mm. of the growing point. (Fig. 2, D.) Both its radial and cross walls become heavily suberized (fig. 2, A) and form a 4-cornered frame which is firmly wedged in the cell and serves to a certain degree as support. On the inner tangential face of these cells, i. e., the wall facing the endodermis, a netlike mesh is developed. (Fig. 2, B.) Occasionally an incomplete net is formed on the opposite wall. This heavily suberized cortical layer completely surrounding the endodermis forms an important protective sheath (17).

Both this layer and the endodermis differentiate at about the same time. The radial thickenings of the former are much more pronounced than those of the latter. The identity of the endodermis is determined by its location, immediately outside the pericycle, rather than by the small inconspicuous strips radially placed. The cortex extends posteriorly for a distance of 2 to 3 cm. Its cells enlarge somewhat as the root matures. The outer vacuolated ones soon collapse and pull apart (fig. 2, A); the inner ones are stretched and torn, and gradually the whole tissue is detached.

The pericycle of the stele is a single-layered concentric ring next to the endodermis. Its rectangular cells are small in cross section. (Fig. 2, C.) From meristems arising in it apparently opposite the xylem points the lateral roots originate. As these cells increase in number and size the walls of the endodermal and cortical cells are pushed apart. Their suberized thickenings form a protective coat about the developing rootlet.

Differentiation of the primary vascular tissue begins 2 to 3 mm. behind the growing region next to the pericycle. The sieve tubes of the protophloem are the first to differentiate. (Fig. 1.) Alternating with the two primary phloem groups, the elements of the two primary

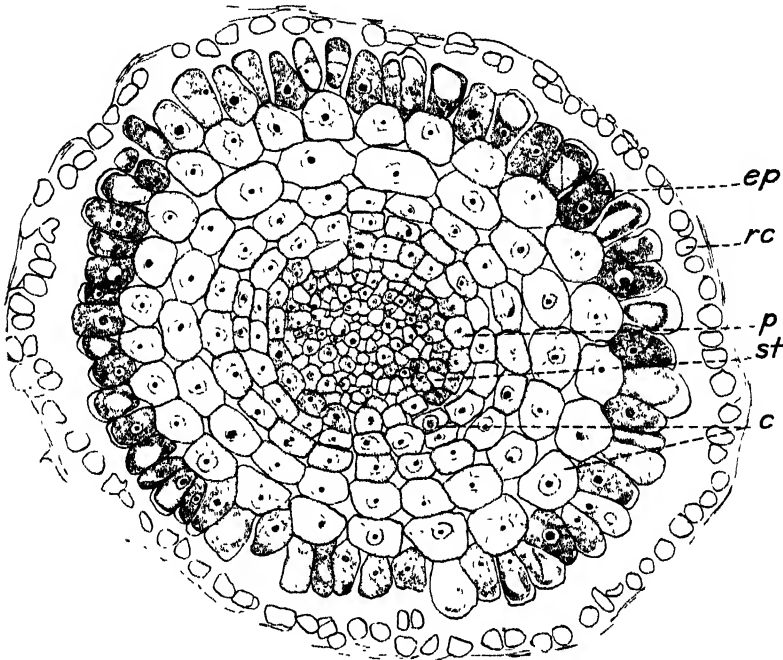


FIGURE 1.—Cross section through the primary meristem of a young cabbage seedling root: *rc*, Rootcap; *ep*, forerunner of epidermis; *c*, forerunner of primary cortex, four and five layers deep; *p*, pericycle; *st*, sieve tube. The primary phloem, but not the primary xylem, has begun to differentiate. \times about 200

xylem groups next become distinguishable. (Fig. 2, C.) The protoxylem groups progress centripetally (fig. 2, D and A) until they meet to form the xylem plate. From then on increase in the primary xylem takes place to the right and left of the plate.

Secondary growth is initiated when the seedling is about 3 weeks old, growing at a soil temperature of about 24° C. under greenhouse conditions. Bands of meristem arising between the primary xylem and phloem and gradually extending completely around the xylem cut off secondary tissues. Simultaneously with the increase in the diameter of the stele the cortex is slowly loosened and finally sloughed off. Tangential division of the pericycle gives rise to a secondary protective tissue, the periderm.

EFFECT OF SOIL STERILIZATION ON THE ROOTS OF CABBAGE

The development of the fungus subsequent to penetration was studied in plants grown upon infested soil. In order to eliminate as

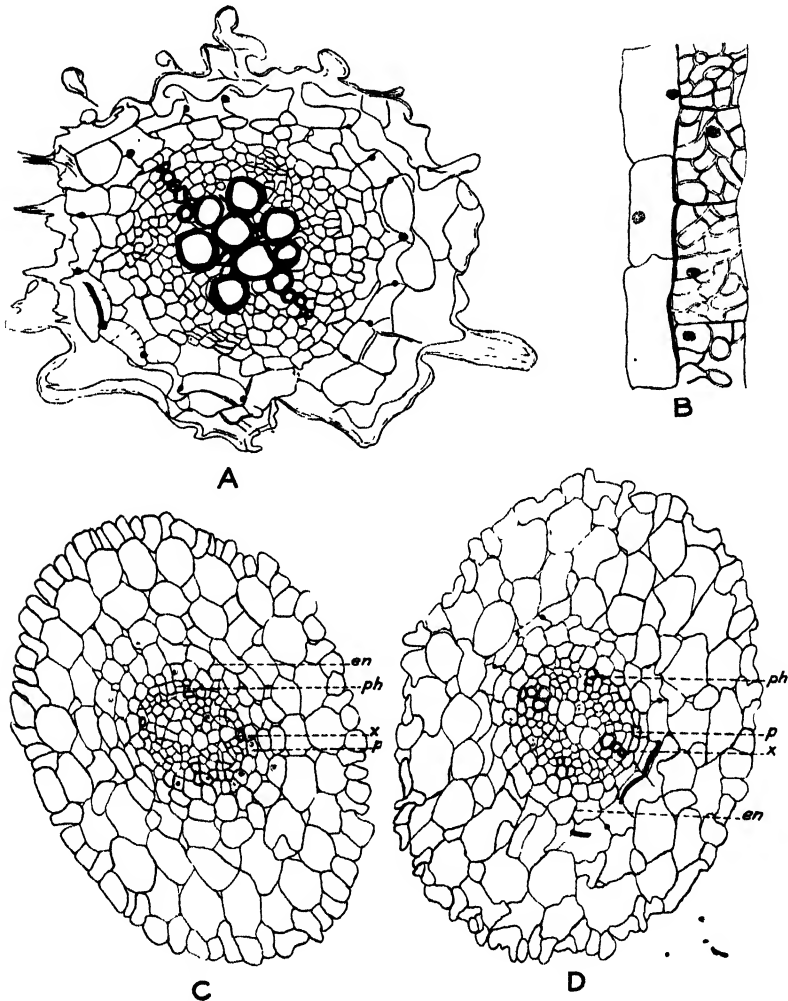


FIGURE 2—A, Cross section of a young cabbage-seedling root in the stage where the epidermis and the outer cortex are collapsed. The cortical layer surrounding the endodermis shows its characteristic thickenings in both cross and radial walls. $\times 173$. B, Tangential longitudinal section of the cortical layer surrounding the endodermis. Note the characteristic net on the wall facing the endodermis. The section passes through the suberized thickening of one of the radial walls. $\times 473$. C, Cross section through the zone of maturation in a young root. *en*, endodermis, *p*, pericycle; *ph*, phloem; *x*, xylem. The cells of the epidermis and cortex are vacuolate, the radial walls of endodermis are slightly suberized, and the primary xylem points have been initiated. $\times 173$. D, Cross section of slightly older region of the same root. Some cells of the cortical layer surrounding the endodermis have suberized cross and radial walls. The abbreviations have the same meaning as in C. $\times 173$.

far as possible the presence of other soil fungi, sterilized soil inoculated with the causal organism was used. This introduced the complication of injury to the root system due to toxins released by heating the soil. In order to understand the nature of this injury a study was made of

the roots of plants (C-16 \times 31B) which had grown from seed for one month upon recently sterilized soil and upon unsterilized soil.

The roots of seedlings grown in freshly sterilized soil and examined three weeks to a month later exhibited different degrees of injury. Many of the very young ones were more or less discolored, varying from a dirty-white or yellow to a yellow-brown gum color; the older roots were less yellowed. The cortex of deeply yellowed roots was somewhat collapsed, and occasionally the growing region had disintegrated. Microscopic examination of the fixed sections of these same roots often showed that the epidermis had disappeared and that the walls of the outer cortex were thickened. In some instances a few of the vessels were filled with a gumlike substance. Young roots slightly discolored and older roots showing little or no discoloration seldom showed this response.

The roots of seedlings grown on unsterilized soil were decidedly white and seldom exhibited any discoloration whatsoever. A microscopic examination of young roots showed that the walls of the epidermis and cortex were normal in thickness and that there was a total absence of plugged vessels.

In the light of this evidence it became important not to confuse the effects of soil toxins with the possible effects of the invading fungus upon the host cells.

RELATION OF FUSARIUM CONGLUTINANS TO SUSCEPTIBLE STRAINS OF CABBAGE

Very little histological work has been reported on the relation of *Fusarium conglutinans* to its host. Gilman (5) isolated small amounts of the fungus from the stems of cabbage after their leaves had become markedly yellowed. He found the hyphae and conidia confined to the xylem in the living host but after its death traversing all tissues and sporulating both within and at the surface. He believed that it attacked the roots first. Tisdale (13) observed the hyphae entering the root hairs of cabbage seedlings grown on artificial media. Both these workers presumably used commercial susceptible varieties, but they made no definite statement in regard to this point.

The results of the present study will be discussed (1) in relation to penetration by the fungus and (2) in relation to its subsequent development in the susceptible host. The drawings, unless otherwise specified, are from preparations of VRC.

PENETRATION

Studies of fungal penetration were made with seedlings grown at a temperature of 24° C. on inoculated soil-extract agar in the manner previously described. The plants were examined at 24-hour intervals, and the seedlings were fixed one to eight days after inoculation. Microscopic examination of the plates 48 hours after inoculation showed the fungus growing plentifully on the agar. Many of the hyphae wove themselves around the root hairs, some were in contact with the roots, and still others were seen penetrating the embryonic tissues.

More than 200 roots inoculated in the region of the root hairs were fixed and examined. In no instance was the fungus observed within a root hair filled with protoplasmic contents. Occasionally it was

seen in older hairs more or less devoid of contents and somewhat shriveled. (Fig. 3, A. D.) Penetration by way of the root hairs in the roots examined was so infrequent that it was of no consequence

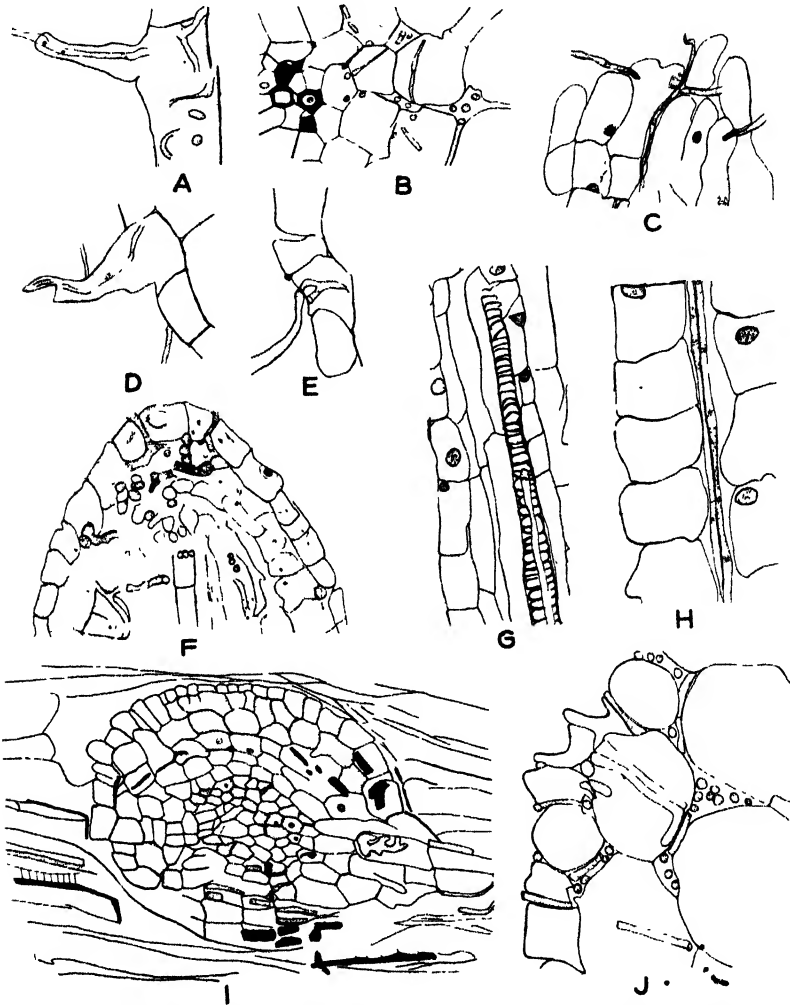


FIGURE 3.—Studies of penetration of *Fusarium conglutinans* in commercially susceptible and in homozygous susceptible cabbage grown at 24° C. on inoculated soil-extract agar. Fixations made three to eight days after inoculation. A, Fungus in an epidermal cell and its root hair. $\times 473$. B, Fungus in cortex and stele behind the impediment of wound response $\times 217$. C, Hyphae in the intercellular spaces of rootcap. $\times 578$. D, Hypha in root hair (Preparation from C-16 $\times 31B$.) $\times 473$. E, Hypha entering epidermal cell $\times 473$. F, Hyphae entering and within primary meristem. $\times 473$. G, Hypha within the latest differentiated tracheal vessel eight days after inoculation $\times 473$. H, Longitudinal section of primary meristem in the region which later differentiates into cortex. Hyphae intercellular. $\times 473$. I, Hyphae in both primary and secondary roots. The indications are that the fungus passed from the main to the lateral root. The heavy dark areas represent suberized walls, peculiar to cortical cells in the young root of cabbage. $\times 217$. J, Cross section of a young root. Fungus both intercellular and intracellular. (Preparation from C-16 $\times 31B$.) $\times 473$.

whatsoever. Tisdale (13) reported penetration of cabbage root hairs by *Fusarium conglutinans* in young seedlings grown in tube culture of potato agar, but he did not state how frequently it occurred.

The most important channels of fungal penetration are the embryonic region and the zone of elongation. Very soon after inoculation hyphae push their way in and out of the intercellular spaces of the rootcap and enter the primary meristem. (Fig. 3, C, F.) Infrequently the fungus enters an epidermal cell directly. (Fig. 3, E.) Behind the region of active cell division the cells of the meristem become vacuolated and enlarged, particularly in their dimension parallel with the length of the root. The fungus has a longer time to attack this part of the root and is often rather abundantly distributed in its cells and in the interspaces of the zone of elongation. Figure 3, J, shows the hyphae within the cells and in the intercellular spaces. In a longitudinal section (fig. 3, H) the hyphae are shown making their way unimpeded for a considerable distance.

Fungal growth upward in the parenchyma of the older cortical tissue might facilitate infection of lateral roots by bringing hyphae in contact with emerging tips, but such a method has not been observed. When the lateral root breaks through the primary cortex, the hyphae occasionally pass into the root rupture and invade the matured cortex of the main root. Fungal penetration in this region, however, is of little importance. The heavily suberized walls of the inner cortical cells are an effective barrier to its inward progress, and in the natural sequence of development the parenchymatous cells of the cortex soon slough off. There is a possibility that the fungus may pass from the main root to its lateral. (Fig. 3, I.)

Fusarium conglutinans is a vascular fungus and makes its way into the protostele while that tissue is still undergoing differentiation. (Fig. 3, G.) After the innermost cortical cells become suberized the stele is barricaded and further radial ingress of the fungus is thwarted. Once within the vascular region, the fungus is more or less confined to the xylem and pushes into the aerial parts of the plant. In a few instances conidia were found in the vessels.

Cabbage is usually started in seed beds and transplanted into the field when 4 to 6 weeks old. During the transfer rootlets are frequently broken or injured. In an experiment to determine whether the fungus could penetrate injured tissue, the tips of seedlings grown under aseptic conditions were cut off. Some of these were placed in Petri dishes of soil-extract agar with inoculum directly in front of the cut portion. The checks were not inoculated. Three to five days later the exposed cells of cut ends of both inoculated and uninoculated roots were more or less collapsed and dead. Stained sections of fixed material showed the walls somewhat thickened, and both walls and lumina stained heavily with safranin. Occasionally, the fungus passed the impediment of wound response and entered the cortex and stele. (Fig. 3, B.)

In the penetration studies of cabbage seedlings grown on inoculated soil-extract agar the most important places of fungal entrance are the embryonic region of the root by way of either the rootcap or the outermost layer of cells of the primary meristem in the zones of active cell division and elongation. In addition, at times, the fungus may enter injured or broken roots, and infrequently makes its way in by the root hairs. The method of entrance is usually intercellular and infrequently intracellular.

SUBSEQUENT DEVELOPMENT

Further investigations of *Fusarium conglutinans* in relation to its host were continued on somewhat older plants grown in the soil. Seeds of HG-27A were sown in a cool greenhouse in flats of sterilized soil. Thirty days later they were transplanted to cans of artificially inoculated soil and placed in tanks kept at an approximately constant soil temperature. Cabbages grown at 18° to 22° C. exhibited slight yellowing of the lower leaves 22 to 24 days after the transfer. The yellowing became deeper and more extensive and the disease advanced. Then the younger leaves underwent a similar alteration. Four days after the first ill effects were noted some of the plants were dead. Most of them, however, remained alive from a week to 15 days. At 24° to 26° the first disease symptoms were displayed 12 and 10 days, respectively, after transplantation, and the death rate was more rapid than at the lower temperature. At 28° and 30° the plants manifested characteristic disease symptoms somewhat earlier and succumbed more quickly.

When the leaves began to yellow the plants were sectioned and fixed. The primary cortex of the root was frequently yellowed; in advanced stages of disease the vascular systems of the root and stem and occasionally the veins of the petioles and leaves were more or less browned. In general, no appreciable differences were observed in the distribution of the fungus or in its relation to the host in the plants grown at the various soil temperatures. To avoid undue repetition this report is limited to plants grown at 24° C.

The fungus was found to enter through the embryonic structures of the root and to make its way readily into the vascular system of the primary and secondary roots. Commonly, only a few hyphae were discernible, and these were usually confined to the vessels and parenchyma of the xylem. Figure 4, B, shows a longitudinal section of a young root. A hypha is seen entering at *a*, and a number of mycelial threads are distributed in the procumbial region. In a cross section of a young root (fig. 4, A) the fungus is present in the vessels and parenchyma of the primary xylem. In very severe cases of infection the primary tissues as far as the procambium strand were often so disorganized that the tracheal vessels were left bare.

The distribution of the fungus in the young secondary root was similar to that already described for the primary root. In a few of the roots examined the fungus was present in both cortex and stele (pl. 1, A), but more frequently it was confined to the xylem. The hyphae were vigorous; once in a while septations were observed in them, and conidia were sometimes in evidence. (Pl. 2, A, and fig. 4, D.) In such cases the host did not exhibit any unfavorable reactions. In others the vessels were plugged with gummy material, but no definite relationship could be established between the fungus and the occlusions. (Pl. 1, D, and fig. 4, G.)

In the older taproot the invasion was generally more extensive. The fungus had had a longer time to attack the tissues and had been reinforced with mycelial threads from the lateral roots. Wood fibers, cambium, secondary xylem, and phloem had been differentiated. In many of these roots the fungus occurred in the xylem only. At times, however, in very severe cases of infection hyphae were present in all tissues of the stele. (Fig. 4, C.) Although most of the hyphae ran

parallel to the length of the root they sometimes made their way radially from one parenchymatous cell to another (fig. 4, E) or passed through the pits of reticulated vessels (fig. 4, F). Plugging of vessels in the older root did occur, but it was infrequent and could usually be traced to occluded vessels of the secondary root.

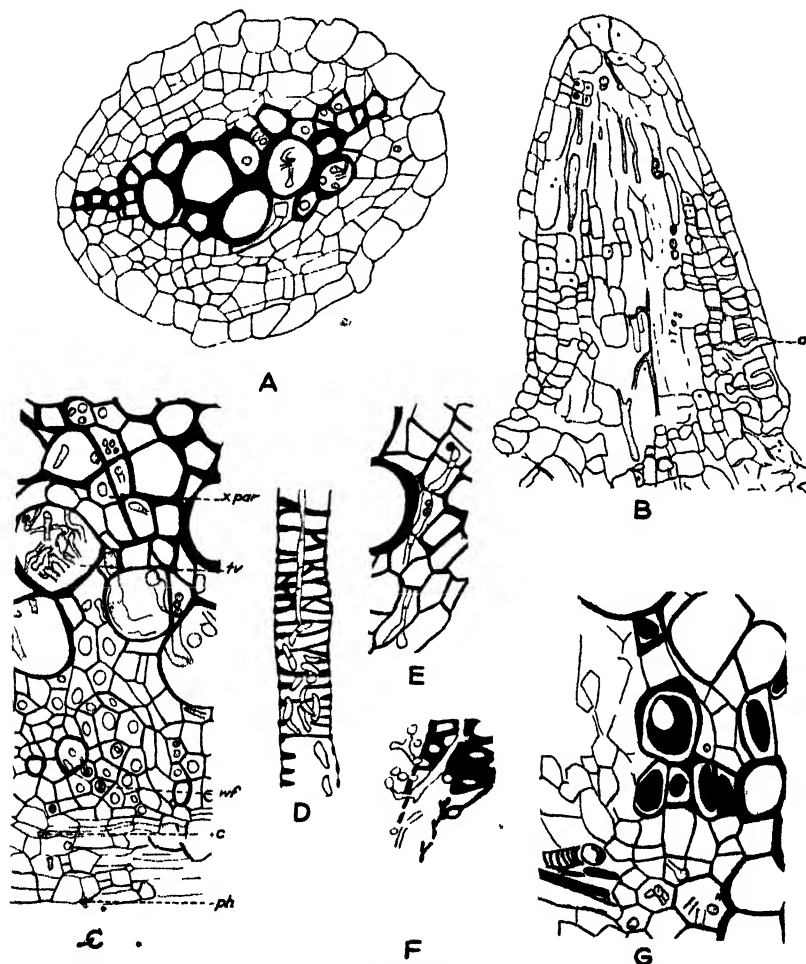
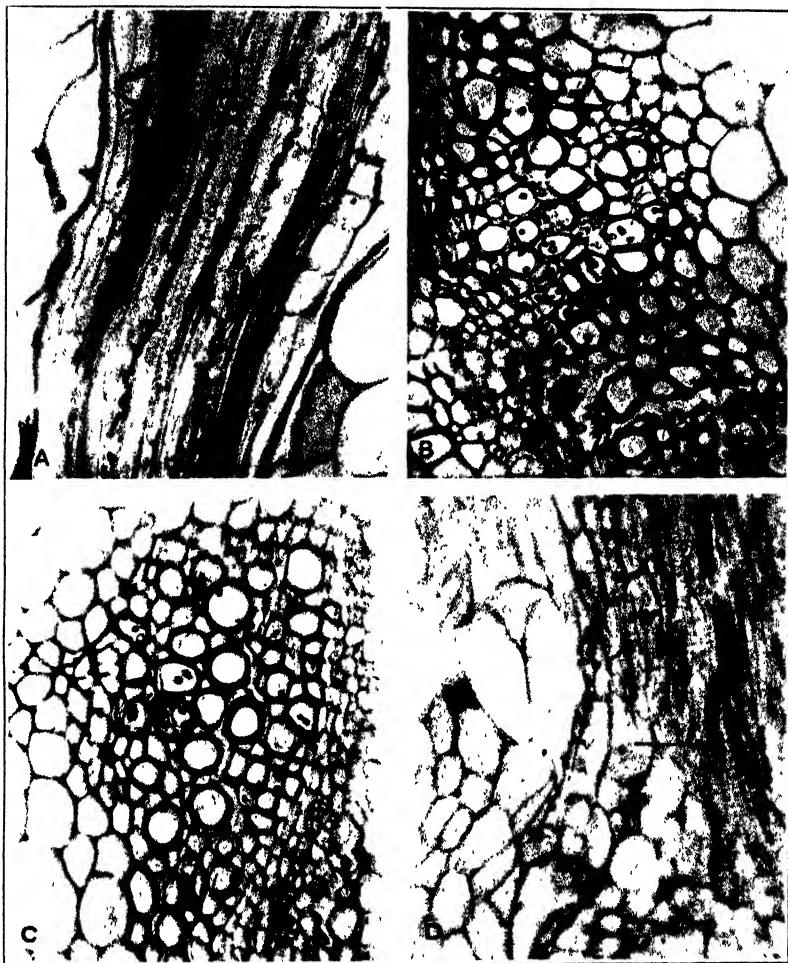


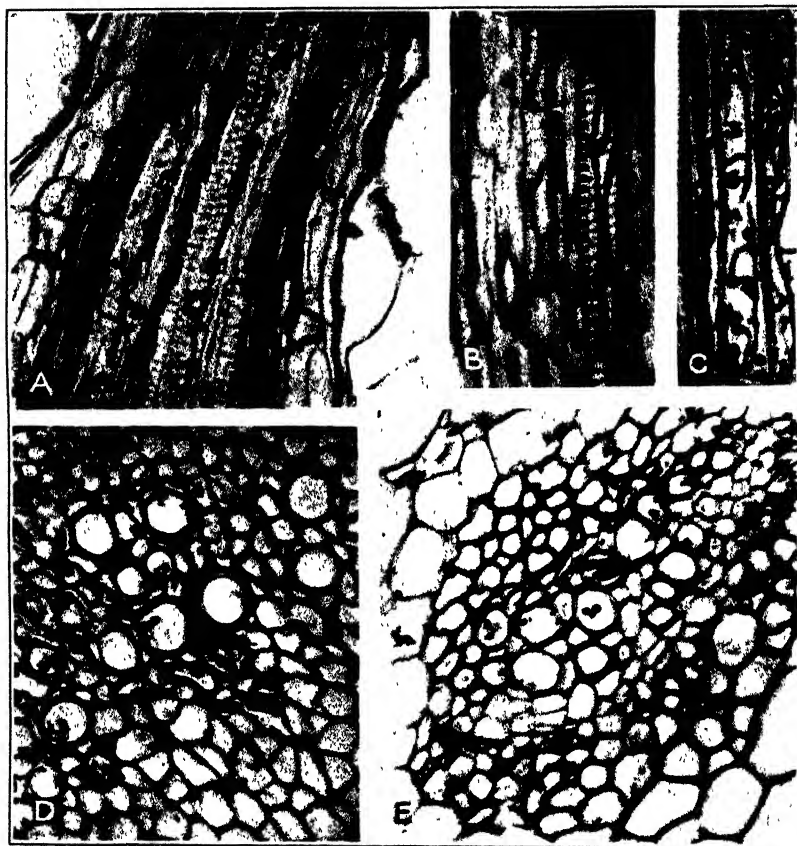
FIGURE 4.—Homozygous, susceptible cabbage transplanted to inoculated soil maintained at soil temperature 24° C. and fixed 12 to 18 days later: A, Cross section of a young root. Fungus in both vessels and parenchyma of the primary xylem. $\times 473$. B, Longitudinal section of a young root. One hypha entered at *a*. Hyphae distributed throughout the primary meristem. $\times 217$. C, Cross section of an older root in which secondary differentiation has taken place. Hyphae in *x par*, xylem parenchyma; *t v*, tracheal vessel; *w f*, wood fiber; *c*, cambium; and *ph*, phloem. $\times 473$. D, Conidia in longitudinal section of a tracheal vessel. $\times 473$. E, Hyphae making its way radially in the xylem parenchyma. $\times 473$. F, Hypha passing through pit of a reticulated vessel. $\times 473$. G, Cross section of a taproot. Some of the vessels are plugged with a gumlike substance; some are invaded by the fungus. There is no apparent relationship between the fungus and the occlusions. $\times 473$

In the upper hypocotyl and in the zone of transition between root and stem, the oldest part of the plant, the relative abundance of the hyphae diminished. Lateral roots were no longer bringing in additional hyphae. The fungus was confined to the tissues of the xylem. Occa-



HOMOZYGOUS SUSCEPTIBLE CABBAGE TRANSPLANTED TO INOCULATED SOIL
MAINTAINED AT A SOIL TEMPERATURE OF 24° C. AND FIXED 12 TO 18 DAYS
LATER. ALL X 100

A, Longitudinal section of a secondary root. Hyphae in both stele and cortex; B, cross section of young stem. Hyphae in both vessels and parenchyma of xylem; C, cross section of stem. Hyphae in both vessels and parenchyma of xylem. The walls of four of the invaded vessels are browned and the lumen of one is half filled with a gumlike substance; D, longitudinal section of a secondary root. Two of the vessels are filled with gumlike substance. There is hypha at the left of the lower occluded mass designated by the arrow.



HOMOZYGOUS SUSCEPTIBLE CABBAGE TRANSPLANTED TO INOCULATED SOIL MAINTAINED AT A SOIL TEMPERATURE OF 24° C. AND FIXED 12 TO 18 DAYS LATER. ALL X 110

A, Longitudinal section of secondary root. Conidia present in the stele; B, the hyphae extend parallel with the length of the vessel; C, both hyphae and conidia are present in the xylem elements; D, hyphae present in the xylem of the stem. Three of the invaded vessels have their walls decidedly browned; E, hyphae are established in the xylem elements of the petiole.

sionally a tracheal vessel stained yellowish red with safranin. This is the characteristic way that browned vessels react to the stain. There is practically a total absence of plugging except in the protoxylem which is being crushed out by the secondary tissues.

Most of the bundles of the stem are free of the organism. In some few, however, it occurs in the tracheae and parenchymatous cells of the xylem. (Pls. 1 and 2.) The walls of some of the vessels are frequently browned in the later stages of disease. Plugging is infrequent and seldom associated with the fungus. Occasionally the contents of one or two near-by parenchymatous cells are granular. (Pl. 1, C.) Relatively few hyphae pass into the veins of the petioles and leaves. (Pl. 2, E.) Plugging of the vessels with gumlike occlusions was occasionally observed in the petioles.

RELATION OF *FUSARIUM CONGLUTINANS* TO RESISTANT STRAINS OF CABBAGE

As already stated, progenies of All Head Early plants homozygous for resistance to *Fusarium conglutinans* were used. The method of studying the fungus in relation to the host is the same as that employed for the susceptible host.

PENETRATION

Seedlings grown on inoculated soil-extract agar at 24° C. were used. In the plate cultures there was no indication that a substance which was antagonistic to the fungus diffused out from the host, inasmuch as the hyphae grew in proximity to the roots, as already described for the susceptible plants. Nevertheless penetration was infrequent, as shown by the complete absence of the fungus in a large percentage of the roots sectioned. An examination of a large number of roots showed that the avenues of entrance were the same as those already described for the susceptible host. Evidence was obtained that the fungus pushed through the intercellular spaces of the rootcap (fig. 5, B), made its way between the epidermal cells (fig. 5, C), penetrated the epidermal cells directly (fig. 5, D), or entered root hairs (fig. 5, A).

The fungus also enters through wounds. The tips of a number of young seedlings were removed under aseptic conditions. Fixations were made three to five days later. The walls and cells of the injured portions stained heavily. Figure 5, J, represents the condition as observed in one inoculated root a short distance behind the incision. Some portions of the section still show the effects of the mutilation. Hyphae are present in the xylem elements.

The root of one seedling fixed four days after inoculation was an exception to the general rule. In the zone of elongation for a distance of about 1 mm. the walls of the cells in the inner portion of the cortex thickened abnormally and stained heavily with safranin. The lumina of some cells were filled with a gumlike substance, but no hyphae were in evidence. Mycelial threads were numerous about the periphery and in some instances pushed into the intercellular spaces of the epidermis and outer cortex. Not much of the invaded tissue was affected by the accumulation of gumlike substances. However, certain unusual reactions of host cells and fungus were noted and are illustrated in Figure 5, E-G. The walls of the cortical cells in a number of successive sections were thickened but no hyphae were in evi-

dence. This reaction in the root can not be explained with certainty. It may be due to the fungus. From the large number of resistant seedlings that did not respond to its presence this does not seem probable. It is more likely that the seedling was injured in transference to the inoculated agar and that the reaction is a wound response.

Generally there is little fungus in the zone of elongation. At times, however, a number of hyphae penetrate the intercellular spaces of the epidermis and enter the parenchymatous cells of the cortex. (Fig. 5, H.) The cell walls are pushed aside by the incoming fungus; but, with the exception cited, they neither thicken nor stain differently from those of the invaded tissue of the susceptible host. There is no accumulation of material in advance of the hyphae either within the cells or in the intercellular spaces. The host is apparently unaffected and the fungus appears normal.

The fungus seldom penetrates the stele of resistant seedlings grown on agar. One seedling fixed four days after inoculation showed a number of invaded vessels, one vigorous hypha extending a considerable distance in a vessel which underwent no apparent alteration. (Fig. 5, I.) The apical part of another vessel in the latest differentiated xylem of the same root was occluded. A mycelial thread was faintly distinguishable within, but it had lost its turgor and was somewhat flattened. Posteriorly in the same vessel it again appeared normal and the passage within the vessel was in no way obstructed. (Pl. 3, C.)

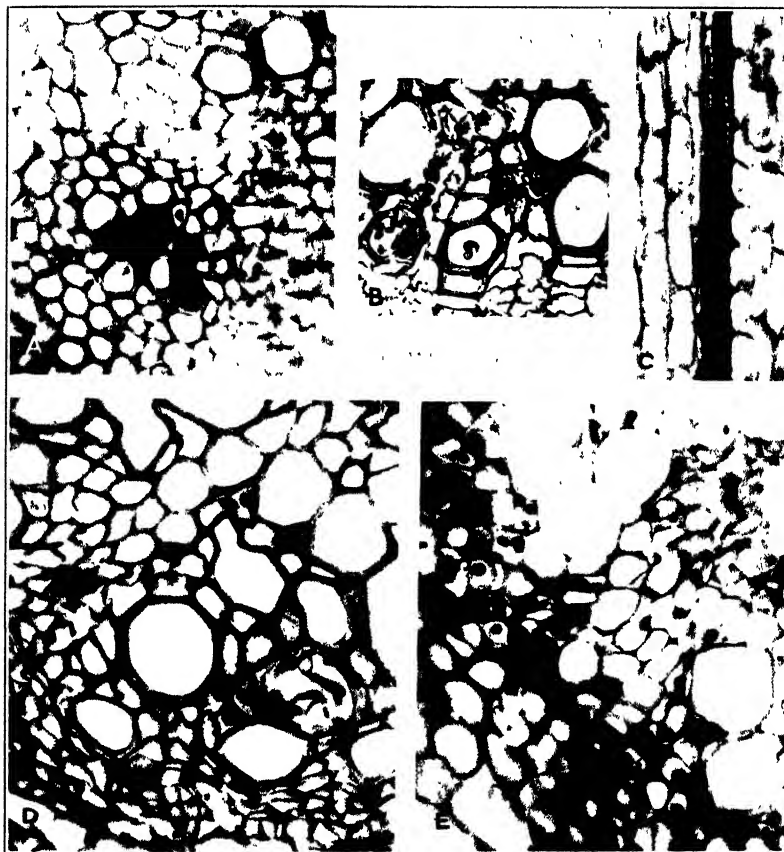
For purposes of comparison, seedlings were inoculated with a second isolation of *Fusarium conglomerans* from Racine, Wis., and with one each from Arkansas and Mississippi. A few hyphae penetrated the interspaces of the epidermis and sometimes invaded the differentiating cortex. No differences were detected in the relationship of the host and the fungus in these isolations and in that of the Racine isolation of November, 1926.

SUBSEQUENT DEVELOPMENT

Further studies of the distribution of the fungus in the resistant host were made with cabbages grown in infested soil. The method of treatment was the same as that already described for the susceptible host. When the seedlings were 1 month old they were transplanted to inoculated soil maintained at the desired temperature. Plants were fixed at various intervals 30 to 40 days later. In the earlier investigations the fungus was not detected in the many plants sectioned and stained. To facilitate the search for the presence of the fungus in the later work, thin slices from each portion being fixed were plated. Those from which the fungus was recovered were dehydrated, infiltrated with paraffin, and sectioned.

The fungus was rarely found in plants grown at soil temperatures below 28° C. and only to a limited extent in those grown at 28° and 30°. In the majority of infected plants studied the fungus was confined to the root; infrequently it extended into the stem. Wherever found, its cells were usually turgid and well supplied with protoplasm.

Most of the plants from which the fungus was recovered in only the roots displayed no external evidence of disease and exhibited no



HOMOZYGOUS RESISTANT CABBAGE. ALL X 160

A-D, Transplanted to inoculated soil maintained at a soil temperature of 28°C . and fixed 30 to 40 days later; A, two vessels and a number of parenchymatous cells in a vascular bundle of the stem plugged. The fungus does not appear to be present, B, the contents of two parenchymatous cells which contain hyphae are quite granular, C, an abnormal hypha is faintly distinguishable within the occluded vessel; D, the contents of the parenchymatous cells immediately surrounding the invaded trachea stain somewhat deeply. The unfavorable reaction may be due to the presence of the fungus, E, grown on inoculated soil-extract agar at 24°C . and fixed 8 days later. The fungus is abundant in the stele of an older root. The host shows no unfavorable response

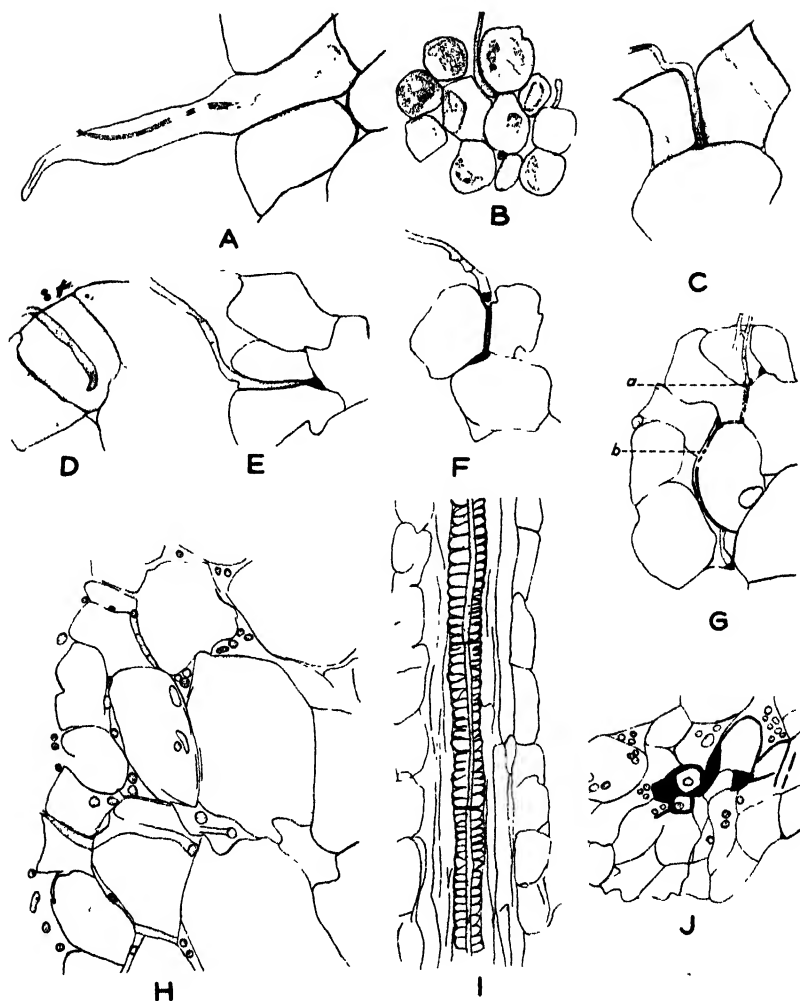


FIGURE 5—Studies of penetration of *Fusarium conglutinans* in homozygous-resistant cabbage grown at 24° C. in inoculated soil-extract agar. Fixations made three to eight days after inoculation. A, Hypha in root hair. $\times 473$. B, Cross section of rootcap. Hyphae in the spaces between cells being sloughed off. $\times 473$. C, Hypha entering intercellularly. $\times 473$. D, Hypha that has penetrated cell of outermost layer of primary meristem. $\times 473$. E–G, Sections of a root in which there are evidences of disturbance. $\times 473$. E, Hyphae passing between two cells of the outermost layer of primary meristem and in contact with dark-staining mass. F, The hypha still outside the host. Its tip stains heavily and the walls of the cells in front of it are swollen and laminated. G, A deep-staining substance is deposited on each side of the hypha at a, where it meets the inner wall of the outermost layer of the primary meristem; at b the wall is thickened; however, the fungus is in contact with only a small part of it. H, Cross section of a young root. Fungus both intercellular and intracellular. $\times 473$. I, Hypha within the lumen of a differentiated tracheal vessel. $\times 473$. J, Fungus in cortex and stele behind the impediment of wound response. $\times 217$.

symptoms of browning. Very little fungus occurred within any of them. At times it was recovered in platings, but it could not be detected in the fixed section from which the plating was made. In others one or more vessels contained a few hyphae and at times the parenchyma was invaded to a slight extent.

Various evidences of disturbance within the host were found, but none of them occurred uniformly. Invaded vessels were usually not obstructed by any gumlike substance, but at times the hyphae within seemed to form a conglomerate mass, and in a few instances gumlike deposits were associated with the pathogene. The contents of parenchymatous cells surrounding them sometimes stained more heavily or were more granular than those of neighboring cells, suggesting possibly that their protoplasm had responded to the presence of the fungus. Parenchymatous cells in which the fungus was estab-

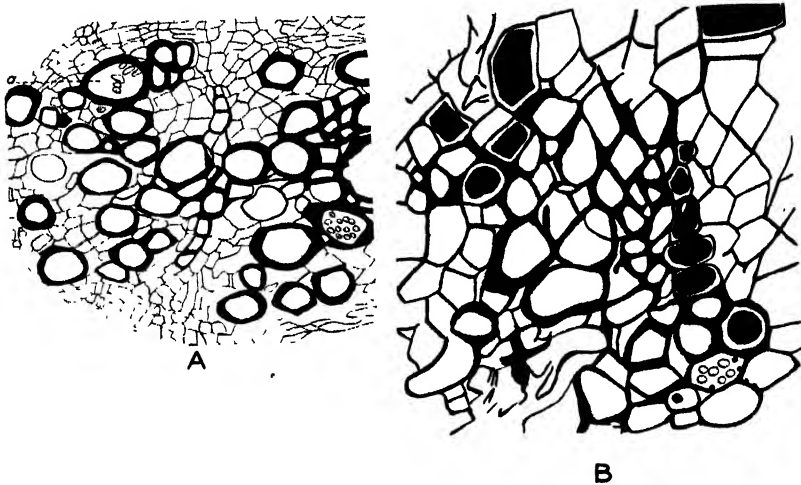


FIGURE 6.—Homozygous-resistant cabbage transplanted to inoculated soil maintained at a soil temperature of 28° C. and fixed 30 to 40 days later. Cross sections of young roots. A, Hyphae occupy one vessel of each of the xylem groups. The contents of two of the parenchymatous cells surrounding *a* are slightly granular. Note that none of the vessels are plugged with a gumlike substance. $\times 217$. B, Cross section of a young root. Hyphae and deposits or disintegrated hyphae are present in one of the vessels. Note that the vessels of both primary and secondary xylem are plugged but that no hyphae are evident in the gumlike mass. $\times 473$

lished usually contained but one hypha and underwent no apparent change. In some roots there was no indication of plugging; in others one or more of the xylem elements were filled with the gumlike substance. (Fig. 6.)

When the invasion was more extensive at the time of fixation, the plants often appeared somewhat stunted and the leaves showed atypical symptoms described elsewhere (15). Browning was present in the walls of some of the tracheal and of some xylem parenchyma cells of both root and stem. Sometimes a large part of the young taproot was disorganized and hyphae permeated all its tissues. But in the older portions of the same root where the tissues were intact they were confined to the vascular system. (Pl. 3, E.) If disturbances appear within the host they are confined largely to the growing portions of the stem. Sometimes the parenchymatous cells surrounding vessels in which the fungus is established stain more deeply than those of

neighboring cells. (Pl. 3, D.) Occasionally the contents are quite granular and massed about the hypha within. (Pl. 3, B.) At still other times the vessels in some bundles are more or less plugged in the apparent absence of the fungus. The case shown in Plate 3, A, is typical of such a condition.

DISCUSSION

The primary purposes of this investigation were to determine whether there were any apparent morphological differences between the cabbages resistant and those susceptible to *Fusarium conglomerans* and whether there were any visible reactions to the presence of the pathogene which might give a key to the basis of resistance.

Browning of vascular elements as previously reported by Jones and Gilman (7), Melhus, Erwin, and Van Haltern (10), and others is a characteristic symptom of the later stages of yellows, but it is probably in no way related to disease resistance. In the incipient stages and in cases of slight infection it is either absent or inconspicuous. Only in advanced stages of disease, such as are common to the susceptible host, does it become noticeable. At times it advances somewhat ahead of the fungus. It seems to be a response of the host to the presence of the pathogene. As is to be expected, if its formation is dependent upon the fungus it is more marked in the susceptible host.

As already noted (15), in the case of resistant plants grown at higher temperatures some browning of the vascular system is common in root and stem. In the majority of such cases the fungus was not recovered by plating. Rosen (11) has suggested in the case of cotton wilt that the fungus might produce in the soil toxins which, being absorbed by the plant, might cause browning without invasion by the parasite. In the case of resistant cabbage it is probable, judging from the histological evidence presented in this paper, that at high temperatures there may be some invasion of the root tips without further advance into the xylem. It is possible that under such circumstances the excretory products of the fungus may be carried up the xylem and result in discoloration of the latter.

The plugging of vessels and of parenchymatous cells with a gumlike substance has been reported by Jones (6) in the winter injury of alfalfa, and in the *Fusarium* wilt of cotton Fahmy (4) noted its occurrence in some of the smaller cells near the invaded xylem cells. Under a number of circumstances it may occur in cabbage in the absence of *Fusarium conglomerans*. Exposed cells of cut roots and more recently differentiated elements of the roots grown in sterilized soil often contain these waxlike deposits which may be the product of the coagulation of cell contents and of a protoplasmic response in adjacent living cells. Protoxylem elements in the region of transition between root and stem, and leaf traces in the normal cabbage sometimes have their passages more or less obscured because of the pressure exerted upon them.

In the cabbage seedlings grown on inoculated agar, plugging was observed in some of the invaded vessels of the resistant but not at all in the susceptible host. The comparison was limited, however, to a small number of roots in which the infection extended into the xylem. In the older cabbage the fungus is largely confined to the vessels and parenchyma of the xylem. Sometimes occlusions of gumlike material

block these structures, particularly in the younger parts of the stem and root. Observational evidence indicates that it is more frequent in the resistant than in the susceptible host grown in infested soil.

The deposit is undoubtedly a response of the host, probably of living cells. Its occurrence in the resistant seedlings grown on agar suggests the possibility that it may halt the progress of the fungus, but the fact that it occurs in the young roots and stems of both homozygous resistant and susceptible lines weakens this assumption. The evidence taken as a whole rather suggests that different disturbing agents may cause injurious effects which promote a similar response and that the accumulation of this gumlike substance is an after effect and does not necessarily represent a form of resistance.

Fusarium conglutinans seldom gains entrance into the homozygous-resistant strains of cabbage, but when it does so it enters by the same routes as in the homozygous-susceptible lines. The avenue in each is largely by way of the intercellular spaces between the outermost cells of the embryonic portions of the root. The absence of direct penetration of active cells and root hairs may be due to their turgidity or to the fact that their distended walls offer no place of lodgment for the hyphae or that their contents are unsatisfactory for the fungal requirements.

It seems hardly possible that resistance is bound up with any volatile substance emitted by the host, for hyphae develop plentifully about the seedlings grown on inoculated agar, but only occasionally penetrate. When they do enter there is, in general, no visible response of the host, which accounts for their sparsity. Evident morphological differences between the resistant and the susceptible lines which seemed to be responsible were not detected by either Tims (12) or the writers.

When cabbage roots are mechanically injured the living cells that are severely affected die. In general, the walls of the cells in the immediate vicinity thicken and the wounded portions heal over. Occasionally, in both resistant and susceptible strains of the host, the fungus was found established in the xylem beyond the barrier, but no differences in the response of the two types of hosts were observed. In an experiment reported in another paper (15, Table 6), the roots were severely pruned and then transplanted to infested soil. Forty-four days after transplantation the majority of the susceptible plants had succumbed to yellows, while the resistant plants showed no symptoms of disease. This brings out the fact that resistance is not confined to the embryonic portions of the root and even though the fungus may enter through wounds it does not thrive in the resistant host.

In a few instances hyphae contained yellow to brown granules, which might suggest a possible detrimental effect of host substance upon the fungus. However, they are relatively rare and are found fully as often in the susceptible as in the resistant plant.

Considerable comparative study has been made of the cell walls of plants which have strains that are susceptible and resistant to parasitic soil fungi. Tisdale (13) working with *Fusarium lini* of flax noted that it penetrated the cortical parenchyma of the resistant plants and that it stimulated cell division and cork formation in cells adjacent to those attacked but that the fungus was not able to invade the tissues to any extent. In a histological study of resistance of tobacco to *Thielavia basicola*, Conant (1) reported that under the influence of

fungal stimulation all secondary tissues of the roots of resistant plants except dead xylem elements could produce cork cells by developing a phellogen in advance of the invasion. In a cytological study of a strain of corn susceptible to *Gibberella saubinetii* grown at 12° C. on agar and inoculated at definite points on the mesocotyl, Pearson⁵ found that the fungus entered through root ruptures, and although the walls of cortical cells in contact with it thickened and gumlike deposits formed in advance of it, the pathogene made its way readily through the cortex. Resistant strains grown at 12°, a temperature favorable for the development of the disease in the susceptible host, are highly resistant to the organism (3). By means of microchemical tests Dickson and Holbert (2) analyzed the nature of this resistance. They found that the cortical walls of the mesocotyl in the susceptible strain grown at 24° and of the resistant strain at 12° and 24° each formed cellulose walls impregnated with suberin, whereas those of the susceptible seedling grown under unfavorable conditions at the lower temperatures did not have walls of true cellulose but were made up of intermediate pectinlike substances which the parasite was able to dissolve and utilize as food.

The investigations reviewed were all made with tissues in which the cells were relatively more mature than that region of the cabbage root through which *Fusarium conglutinans* enters, where the tissues are largely embryonic and the cell walls are in process of formation. The parasite grows through this region and makes its way to the differentiating tracheal vessels. All the evidence indicates that resistance to the pathogene exists even in the youngest portions of the roots of the homozygous host. If this is the case, resistance in the actively growing region must be almost entirely bound up with the protoplasm. Through selection of resistant strains, physiological qualities or chemical substances antagonistic to the fungus have undoubtedly been increased or have been uniformly distributed throughout the cell.

In general, under greenhouse conditions, browning of the vascular elements is a characteristic symptom of the later stages of disease produced by *Fusarium conglutinans*. While plugging of the xylem with a gumlike substance does not occur uniformly, it seems to be a response of the host to the injurious effects of the fungus. The cytological studies made show that resistance is the result neither of morphological differences between the resistant and susceptible hosts nor of visible reactions produced by the resistant host to the presence of the parasite. Since it is present even in the meristematic region of the root where cell-wall differentiation is incomplete it may be due to the cell contents that have been altered by selection and that are adverse to fungal development within the host.

SUMMARY

Fusarium conglutinans makes its entry into the root of the cabbage intercellularly and occasionally intracellularly in the meristematic regions of the root, i. e., through the growing point and the zone of elongation, and infrequently through root hairs. In injured roots it enters through both meristematic and permanent tissue. Its progress through the root and stem is largely confined to the xylem.

⁵ PEARSON, N. L. THE PARASITISM OF GIBBERELLA SAUBINETII. [Manuscript in preparation.]

The fungus readily enters homozygous susceptible cabbage seedlings grown at 24° C. on inoculated soil-extract agar, but it penetrates only occasionally homozygous resistant strains grown under the same conditions.

Cabbage homozygous for susceptibility, 1 month or more old, grown on inoculated soil at temperatures 20°–30° C., is practically a total loss. Cabbage homozygous for resistance, of a corresponding age, grown under the same conditions, is almost completely resistant up to 24°–26°. At 28° and 30° the pathogene is largely confined to the root, but it may rarely extend into the aerial portion and bring about the death of the host.

Resistance to the fungus in strains of cabbage which have proved to be homozygous for resistance to *Fusarium conglutinans* prevails throughout the embryonic and permanent tissues of the root. A comparison of strains of cabbage homozygous for susceptibility with those homozygous for resistance does not in any way indicate that resistance is associated with morphological differences, with visible differences in wall composition, with suberization, with gumlike or other perceptible wall-occluding substances or growths, or with volatile substances. All evidence points to the conclusion that resistance in actively growing tips must be almost entirely bound up with the protoplasm and be attributed to antagonistic chemical substances or physiological qualities.

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A STUDY OF THE ACCURACY OF MEASUREMENTS OF DAIRY CATTLE¹

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INTRODUCTION

One of the principal difficulties in genetic and nutritional experiments with domestic animals is to present in numerical terms an accurate and objective description of the animals at the beginning and during the course of the experiments. Descriptions not expressed in numerical terms are with difficulty, if at all, amenable to statistical treatment. Age and weight partially comply with the requirements for a satisfactory description, and are almost universally used. They are, however, woefully inadequate as a means of describing an animal so that a reader can reconstruct it in his own mind and see it as it actually is. For some animals, such as dairy cows, hens, fine-wool sheep, draft horses, race horses, etc., there are production records which serve as additional and very valuable descriptions. These, however, are incomplete, since animals with equal records of production often differ widely in shape, fatness, quality, and other attributes. Photographs, if taken with due regard to avoiding distortion of perspective and such photographic tricks, are accurate and objective, but it is difficult or impossible to treat them statistically even for such a simple thing as obtaining an arithmetical average.

Measurements of various parts of the animal have been used to a considerable extent as additional means of describing animals and studying changes in size and shape. Their use has been especially common in studies of dairy cattle, but they have also been used for beef cattle, horses, sheep, and even for swine. Since measurements are expressed in numerical terms, they are subject to statistical analysis. If carefully taken, they are almost purely objective, and some are exceedingly accurate. Others depend to a considerable extent upon the position of the animal, and may vary considerably with undetected variations in the pressure with which the operator applies the measuring instruments. Since the validity of the findings is dependent to a considerable degree upon the accuracy of the original measurements,² it was thought worth while to make a study of the extent of error normally encountered in taking measurements. Dairy cattle were used for the study. The results, noted briefly in a study³ of beef steers, are here presented in detail.

¹ Received for publication Sept. 30, 1929, issued June, 1930.

² SHEWHART, W. A. CORRECTION OF DATA FOR ERRORS OF MEASUREMENT. *Bell System Tech. Jour.* 5, 11-26. 1926.

³ LUSH, J. L. CHANGES IN BODY MEASUREMENTS OF STEERS DURING INTENSIVE FATTENING. *Tex. Agr. Expt. Sta. Bul.* 385, 17-18 1928

METHODS OF MEASURING

Nine Jersey cows were each measured 11 times. Twenty-five different measurements were studied. The cow stood on a level scale platform while these were being taken, and one man kept her in what was considered a normal position. Another made the measurements, and a third recorded them. All measurements were made by the same person. After one set was made, the cow was led off the platform and tied in the shade until at least two, and usually three or four other cows, had each been measured once. Then the first cow was led back on the scale platform and the second set of measurements was taken. This was repeated until 11 sets were taken for each cow. The interval between measurements gave sufficient opportunity for the cow to change her position and also for the observer to forget the figures for the previous set. The recorder was careful to make no comment on the previous measurement before the observer had announced the result of the one being taken. Since this work extended over several days, the 11 sets of records for each cow were not all taken on the same day but extended over parts of two or three days in each case, thus giving additional opportunity for temporary variations to be manifested.

EXPERIMENTAL ANIMALS

The cows were selected for measuring with two objects in mind: (1) To obtain a group which was representative so far as age was concerned of the milking herd of the station; and (2) to use where possible cows which were dry or far advanced in their lactation period, so as to interfere little with the milk production of the herd. The cows ranged in age from about 2 years and 4 months to 13 years and 3 months, most of them being near the lower limit. All were purebred or high-grade Jerseys. The measurements were made late in June, 1928.

When the data were being analyzed, the question whether the errors in measuring tended to vary with the size of the animal measured assumed considerable importance, and, in order to get more evidence on this point, 10 heifers were measured in the manner described above, 11 sets of measurements being made on each. This group of measurements was made late in December, 1928, and the time spent in measuring extended over several days. The heifers were selected so as to include all in the herd as near 1 year old as possible. Nine were between 9 and 14 months old, but 1 was only 6½ months old. All were purebred or high-grade Jerseys. The man who measured the heifers had acted as recorder when the cows were measured.

METHOD OF CALCULATION

The method of calculation follows that described by Fisher ⁴ under the general title Analysis of Variance, and is the same as that used in a study of the accuracy of cattle weights ⁵ and in a study of the accuracy of appraisals of dressed beef. ⁶

⁴ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. 230 p., illus. Edinburgh and London. 1925.

⁵ LUSH, J. L., CHRISTENSEN, F. W., WILSON, C. V., and BLACK, W. H. THE ACCURACY OF CATTLE WEIGHTS. *Jour. Agr. Research* 36: 551-580. 1928.

⁶ LUSH, J. L., BLACK, W. H., and SPMLE, A. T. THE USE OF DRESSED-BEEF APPRAISALS IN MEASURING THE MARKET DESIRABILITY OF BEEF CATTLE. *Jour. Agr. Research* 39: 147-162. 1929.

TABLE 1.—Chest-width measurements (in centimeters) of nine cows

Cow No	Measurement No --											Average
	1	2	3	4	5	6	7	8	9	10	11	
41	34.2	31.0	33.1	30.8	30.8	30.9	32.3	31.4	32.8	31.8	31.8	31.90
119	40.8	43.4	42.4	42.1	42.7	41.0	41.9	44.8	42.1	41.8	44.5	42.50
177	40.3	38.7	39.0	39.1	38.5	39.1	37.2	38.4	36.1	41.1	38.2	38.70
184	35.4	37.0	33.6	38.0	34.2	35.6	33.8	33.5	34.0	35.0	35.8	35.08
189	33.6	32.0	33.8	33.5	32.7	32.0	34.1	32.8	35.6	31.8	34.5	33.29
191	36.8	38.2	37.2	36.1	36.8	37.2	35.6	36.7	38.7	36.3	36.2	36.89
193	38.9	37.8	40.9	41.8	36.8	40.7	40.7	36.4	38.8	41.1	36.8	39.43
314	28.1	27.9	28.9	31.3	28.7	27.0	28.5	31.4	28.9	28.6	28.94	28.94
315	33.2	31.3	33.4	32.8	31.8	31.8	32.0	32.6	30.3	33.5	31.1	32.16

Table 1 shows the original data for chest width as obtained for the nine cows from the milking herd. In all, there are 99 measurements in Table 1, and the variations among these 99 items show in general two things: (1) These cows actually do differ from each other rather markedly in chest width and (2) the different measurements taken on the same cow do not agree exactly; that is, there is a certain amount of error in measuring.

TABLE 2.—Analysis of variance in chest-width measurements of nine cows

Cause of variation	Degrees of freedom	Standard deviation	Mean variance per degree of freedom	Total variance
		Centi-meters		
All	98	4.27	18.2014	1,789.62
Differences between cows	8		204.5788	1,636.63
Inaccuracy of measurements	90	1.3	1.6999	152.99

Table 2 shows the calculations for the data presented in Table 1. The total variance (Σd^2) shown by the population of 99 items is 1,789.62. But of this total, 1,636.63 is due to the fact that the averages for the different cows were not identical, that is, to variation between cows in the group chosen for measurement. The remaining variance (152.99) is due to the fact that measurements made again on the same animal did not have exactly the same values. This latter variance may be calculated as indicated in Table 2 or by adding together the squared deviation of each of the 99 measurements from the average measurement for the cow on which it was taken. Since one degree of freedom⁷ is used in fixing the mean for each cow, this total of the intra-class variance is based upon only 90 degrees of freedom. The average standard deviation of measurements taken upon the same cow is, therefore, 1.3 centimeters in the case of chest width. It includes variations due to undetected changes in the cow's position and also to errors on the part of the operator in pressing more firmly with the arms of the caliper at some times than at others. Possible errors in

⁷ By "degrees of freedom" is meant the number of effective items in the population upon which the statistic in question is calculated. Since deviations are figured from the mean and one item of data is required to fix the mean, standard deviations are based in general on one less degree of freedom than there are items in the population. But when, as in this case, deviations are figured from the means of subsamples of a large population one item is used to fix each such mean and the total number of effective items will be many less than the total number of items in the population.

recording and calculating would also be included, although all such work was carefully checked to avoid errors. Corresponding to this standard error of 1.3 centimeters there can be calculated of course a "probable error" of 0.88 centimeter for the accuracy of a single measurement of chest width. These findings indicate that about half of all measurements of chest widths may deviate by as much as 0.88 centimeter more from the true measurements of the cow on which they are taken, even when as many precautions are used to insure accuracy as in these experiments. The general applicability of this finding is enhanced by the fact that the measurements were taken upon nine different cows which presumably differed in the constancy with which they assumed the same position each time they were measured. The general applicability of this finding is slightly lessened by the fact that all measurements were taken by the same operator.

Twenty-four other parts of the body were measured by the method described above, and the data thus obtained were analyzed in the same manner as the measurements of chest width, but the detailed data are omitted from this paper and only the summarized findings are presented. (Tables 3 and 4.)

TABLE 3. —Variation shown by measurements taken on group of nine Jersey cows from milking herd

Measurement	Average standard deviation of single measurements taken on the same cow—		Standard deviation of the mean measurements for each cow	Approximate ratio of variation between cows to error of measurement
	In actual units	As a percentage of the mean measurement of all nine cows		
	<i>Centimeters</i>		<i>Centimeters</i>	
Thickness of skin fold	0.032	3.91	0.10	3
Cannon circumference	.083	.54	.43	5
Length inside ear	.175	1.10	.68	4
Width at eyes	.198	.93	.55	3
Length of head	.223	.49	1.32	6
Width at pin bones	.264	1.09	1.62	6
Width at hips	.31	.67	2.78	9
Width at thurls	.36	.91	1.33	4
Width at loin	.48	1.50	1.92	4
Length of pelvis	.61	1.26	2.07	3
Depth of chest	.67	1.04	3.54	5
Muzzle circumference	.75	2.00	1.53	2
Height at knee	.79	2.38	.95	1
Greatest height at sternum	.90	1.68	2.99	3
Height over withers	.92	.77	3.01	3
Height over hips	1.09	.90	3.30	3
Height at elbow	1.09	1.57	2.01	2
Width at shoulder	1.10	2.89	4.67	4
Width of chest	1.30	3.68	4.31	3
Least height at sternum	1.30	2.52	2.15	2
Heart girth	1.37	.83	11.44	8
Paunch girth	1.78	.87	18.81	11
Flank girth	1.90	1.10	12.51	7
Length of body	2.53	1.80	4.79	2
	<i>Square inches</i>		<i>Square inches</i>	
Dewlap area	2.55	17.21	4.78	2

TABLE 4. --Variation shown by measurements taken on group of 10 Jersey heifers 6½ to 14 months old

Measurement	Average standard deviation of single measurements taken on the same heifer—		Standard deviation of the mean measurements for each heifer	Approximate ratio of variation between heifers to error of measurement
	In actual units	As a percentage of the mean measurement of all 10 heifers		
	<i>Centimeters</i>		<i>Centimeters</i>	
Thickness of skin fold	0.044	4.95	0.21	5
Cannon circumference	.179	1.45	.90	5
Length inside ear	.180	1.25	1.05	6
Width at eyes	.183	1.07	1.25	7
Width at hooks	.187	.63	3.76	20
Length of head	.229	.64	3.02	13
Width at pin bones	.238	1.43	2.48	10
Width at thumbs	.242	.82	3.33	14
Length of pelvis	.279	.78	3.56	13
Depth of chest	.34	.72	4.35	13
Width of loin	.36	1.63	3.22	9
Muzzle circumference	.39	1.33	1.69	4
Width at shoulder	.59	2.36	3.78	6
Width at chest	.67	2.77	3.28	5
Height at knee	.68	2.10	1.49	2
Height over withers	.81	.82	6.76	8
Greatest height at sternum	.92	1.83	2.93	3
Height over hips	.93	.92	6.90	7
Least height at sternum	.98	2.01	2.44	2
Length of body	1.00	.95	10.44	10
Height at elbow	1.01	1.68	3.34	3
Chest circumference	1.13	.93	11.76	10
Flank circumference	1.74	1.41	11.96	7
Paunch circumference	2.01	1.31	11.54	7
	<i>Square inches</i>		<i>Square inches</i>	
Dewlap area	0.50	5.19	2.04	4

DESCRIPTION OF MEASUREMENTS STUDIED

THICKNESS OF SKIN FOLD (fig. 1, S).—This was measured with the small caliper. (Fig. 4.) The measurement was taken over the last rib, a little more than one-third of the distance down from the mid-dorsal line to the mid-ventral line. The skin was pulled out from the rib and the caliper was closed against the skin rather tightly. The measurement thus includes a double layer of skin and hair. The data were used as obtained without adjustment for this doubling.

CANNON CIRCUMFERENCE (fig. 1, V).—The steel tape (fig. 3, D) was pulled tightly around the foreleg at the smallest place between the knee and fetlock joint to obtain this measurement.

LENGTH INSIDE EAR (fig. 2, I).—One end of a steel ruler was placed against the edge of the external opening of the ear nearest the head, the distance measured being from this point to the farthest tip of the ear.

WIDTH AT EYES (figs. 1 and 2, E).—The greatest width of the head at a point about level with the eyes was measured with a small straight-arm caliper. (Fig. 3, C.)

LENGTH OF HEAD (figs. 1 and 2, A).—The extreme length of the head from the highest point on the poll to the end of the muzzle was measured with a small straight-arm caliper (fig. 3, C) in a straight line parallel to the long axis of the front surface of the head.

WIDTH AT PIN-BONES (fig. 2, N).—The calipers (fig. 3, B) were placed against the extreme lateral point of the pin bone (tuber ischii) on one side and the corresponding point on the other.

WIDTH AT HIPS (fig. 2, H).—The caliper (fig. 3, B) was placed on the extreme lateral point of the hooks (ilium) on one side and on the corresponding point on the other side.

WIDTH AT THURLS (fig. 2, J).—The points of the caliper (fig. 3, B) were placed snugly just lateral to the hip joint on each side of the pelvis.

WIDTH AT LOIN (fig. 2, L).—The points of the caliper (fig. 3, B) were placed snugly against the sides of the loin, but no more pressure was used than was necessary to make sure that the caliper points were resting against solid flesh. This measurement was taken midway between the third and fourth lumbar ver-

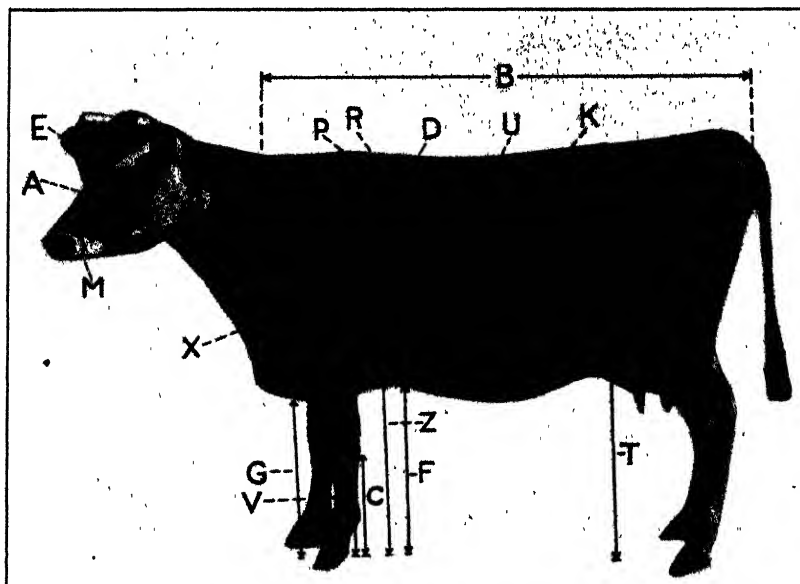


FIGURE 1.—Side view of Jersey cow with diagram showing location of various measurements studied; lettering is explained in the text

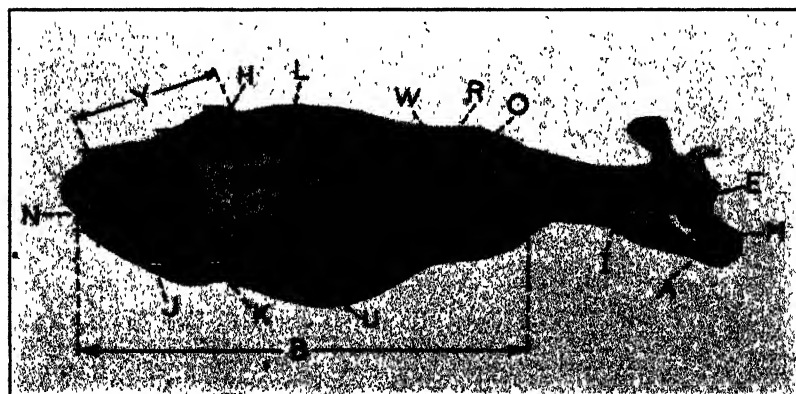


FIGURE 2.—Top view of Jersey cow with diagram showing location of various measurements studied; lettering is explained in the text

tebrae, and therefore about halfway between the forward edge of the pelvis and the rear edge of the last rib.

LENGTH OF PELVIS (figs. 1 and 2, Y).—This was the distance from the extreme posterior point of the pin bone to the extreme anterior point of the hook bone on the same side, so far as that could be located definitely on the live animal. The caliper (fig. 3, B) was used.

DEPTH OF CHEST (fig. 1, D).—This was the smallest vertical outside diameter of the chest and was measured with the parallel bars. (Fig. 3, A.)

MUZZLE CIRCUMFERENCE (figs. 1 and 2, M).—This was taken with the steel tape drawn rather tightly around the nose (including the lower jaw) at the smallest place just back of the nostrils.

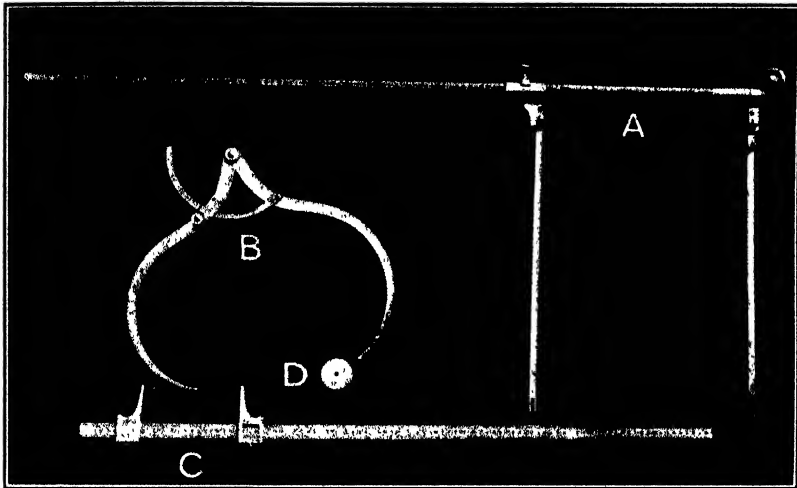


FIGURE 3.—Instruments used in measuring dairy cattle. A, Cattle-measuring standard with spirit level attached. B, calliper used for measurements of pelvic region and for width of loin. C, caliper used for measuring length of head and width at eyes. D, steel tape used for measuring girths and circumferences

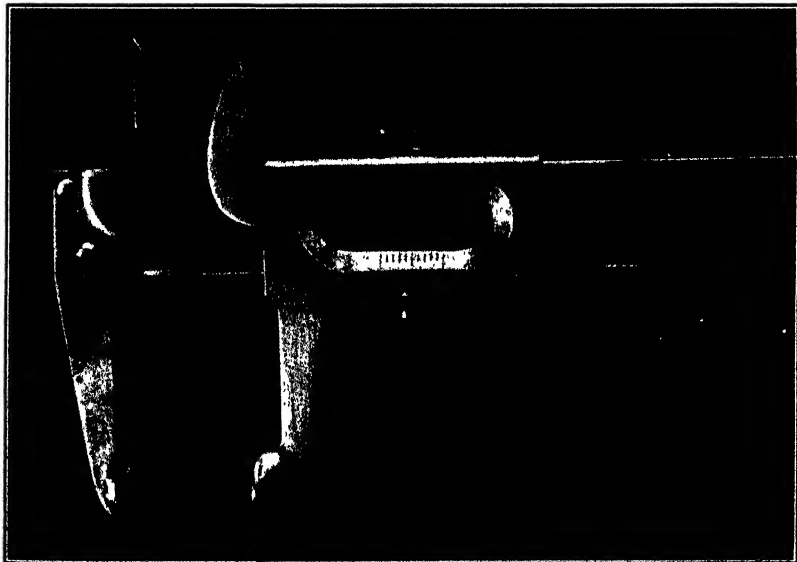


FIGURE 4.—Caliper used for measuring thickness of skin

HEIGHT AT KNEE (fig. 1, C).—This was the vertical distance from the floor to the small bony protuberance on the posterior of the knee-joint (carpus).

GREATEST HEIGHT AT STERNUM (fig. 1, F).—This was the vertical distance from the floor to the highest point on the bottom of the chest.

HEIGHT OVER WITHERS (fig. 1, P).—This was the vertical distance from the highest point over the withers to the ground.

HEIGHT OVER HIPS (fig. 1, T).—This was the vertical distance from the highest point midway between the hooks to the ground.

HEIGHT AT ELBOW (fig. 1, Z).—This was the vertical distance from the ground to the point where the superior and posterior surfaces of the ulna join.

WIDTH AT SHOULDERS (fig. 2, O).—This was the greatest transverse width of the body through the shoulders and was measured at a point 2 to 4 inches posterior to the shoulder joint.

WIDTH OF CHEST (fig. 2, W).—This was the greatest width of the chest just behind the shoulders.

LEAST HEIGHT AT STERNUM (fig. 1, G).—This was the vertical distance from the lowest part of the brisket to the ground. The caliper arm was placed snugly against the solid part of the brisket in such a way that the measurement was not influenced by the amount of dewlap present.

HEART GIRTH (figs. 1 and 2, R).—This was measured with a steel tape drawn snugly around the body in a plane perpendicular to the long axis of the body at its smallest circumference, which is just behind the front legs.

PAUNCH GIRTH (figs. 1 and 2, U).—This was the greatest circumference of the body in any plane perpendicular to the body axis.

FLANK GIRTH (figs. 1 and 2, K).—This was measured with the steel tape drawn snugly around the body at its smallest circumference just in front of the hook bones at the top of the body and as far to the rear as possible underneath the body without including any of the udder.

LENGTH OF BODY (figs. 1 and 2, B).—This was the distance in a line parallel to the main axis of the body from the extreme anterior point of the shoulder point (tuberosity of the humerus) to the extreme posterior point of the pin bone.

DEWLAP AREA (fig. 1, X).—This was the area, seen from one side, of the dewlap. The dewlap is the fold of skin along the mid-ventral line beginning just back of the muzzle and extending far back on the brisket. This area was estimated in square inches by the observer who used a ruler frequently to check his estimates. Since the dewlap area has irregular boundaries, the observer's estimation of it was not as purely objective as the other 24 measurements were.

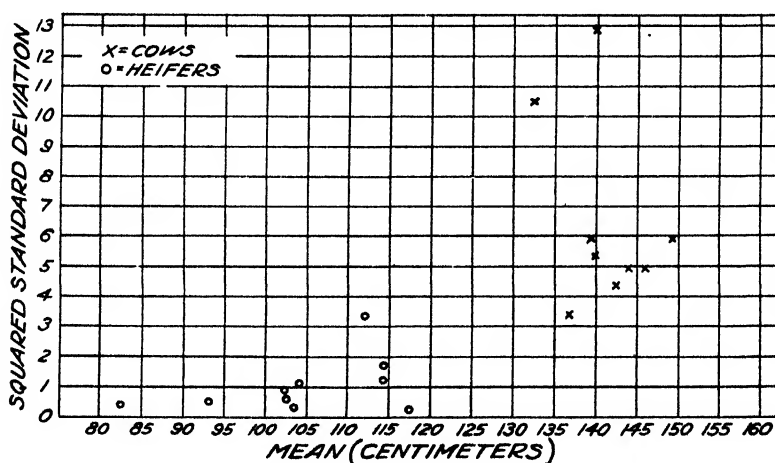
PRESENTATION OF RESULTS

The summarized findings on the group of cows from the milking herd are presented in Table 3 and those from the group of heifers about 1 year old are shown in Table 4. In these tables the error of measuring is presented in actual units in column 2 and as a percentage of the mean of all measurements in column 3. Thus for the cows the standard error in measuring width at eyes is 0.198 centimeter, while the standard error in measuring height over withers is 0.92 centimeter, nearly five times as large. Yet the average width at eyes was 21.28, while the average height over withers was 119.4. Thus the standard error of measuring width at eyes was 0.93 per cent of the mean value of that measurement, while the standard error of measuring height over withers was only 0.77 per cent of the mean value of that measurement. The measurements are arranged in the order of their errors in actual units.

A study (now in press) pertaining in part to skin thickness on beef-bred steers showed standard deviations of 0.09 centimeter in the error of measuring skin thickness and 0.043 centimeter in the error of measuring the thicknesses of hides just after the steers had been slaughtered.

The question whether the error in measuring varies with the size of the measurement, that is, whether it tends to be a certain percentage of the measurement or a definite number of centimeters or square inches regardless of the size of the animal being measured, was studied by means of scatter diagrams made for each measurement. The average size of the measurement for each cow was plotted along one axis and the squared standard deviation of the 11 repetitions of the

measurement on the same cow was plotted along the other axis. Inspection of these diagrams showed clearly that there was for most measurements only a small correlation or no correlation at all between the average size of the measurement and the error in taking that measurement. Figures 5 and 6 show these scatter diagrams for body length and chest width, the two measurements which by inspection seemed to show the highest correlations. Figures 7 and 8 show similar diagrams for heart girth and height over withers which were picked out by inspection as representative of most of the measurements except that they showed a wider range both of averages and of squared standard deviations than most measurements did. Figure 9 shows the scatter diagram for dewlap area which differs from the other measurements in two probably important respects: (1) It is not as purely objective as the others, and (2) the heifers (which of course had smaller dewlaps than the cows even under the same conditions) were measured in cold weather (late December) while the cows were



in the fourth column of Table 3. Column 5 of Tables 3 and 4 is obtained by dividing the figures in column 2 into those in column 4.

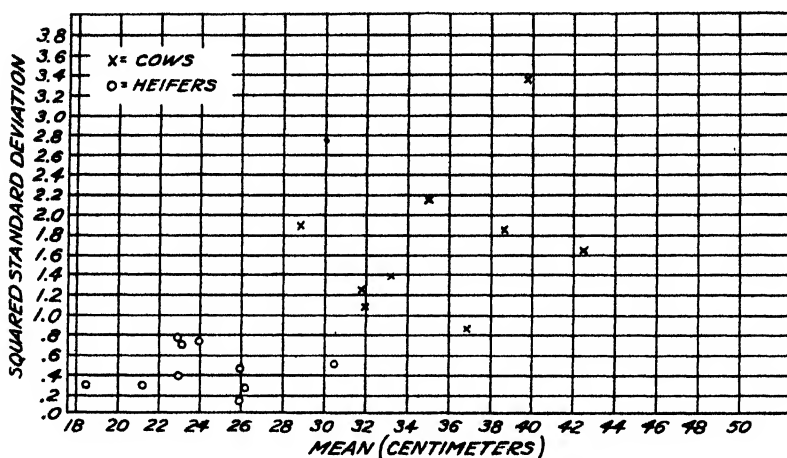


FIGURE 6.—Relation between observed standard deviation and mean size of measurements of width of chest for individual animals

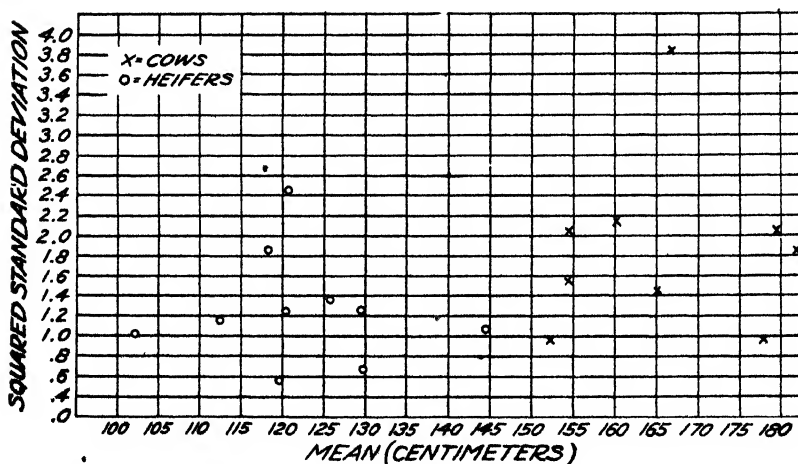


FIGURE 7.—Relation between observed standard deviation and mean size of measurements of heart girth for individual animals

Columns 4 and 5 do not have the general applicability that those in columns 2 and 3 have. The size of the figures in column 4 measures directly the heterogeneity of the group of cows on which these measurements were taken. If the group had been more uniform the figures in columns 4 and 5 would have been smaller, although the figures in columns 2 and 3 would have been changed but little if any. Conversely, less uniform groups of cows and heifers than the ones actually used would have meant larger figures in columns 3 and 4.

The figures in column 4 are not entirely free from the element of error in measuring, even though they are based upon averages of 11 measurements. However, the error of measuring is a very small part

of the figures in column 4. For example, the 0.95 centimeter standard deviation for height at knee is only reduced to 0.92 centimeter when corrected for errors of measurement.⁸ For other measurements the reduction would be relatively less, since the error of measurement is a smaller part of the figure given in column 4.

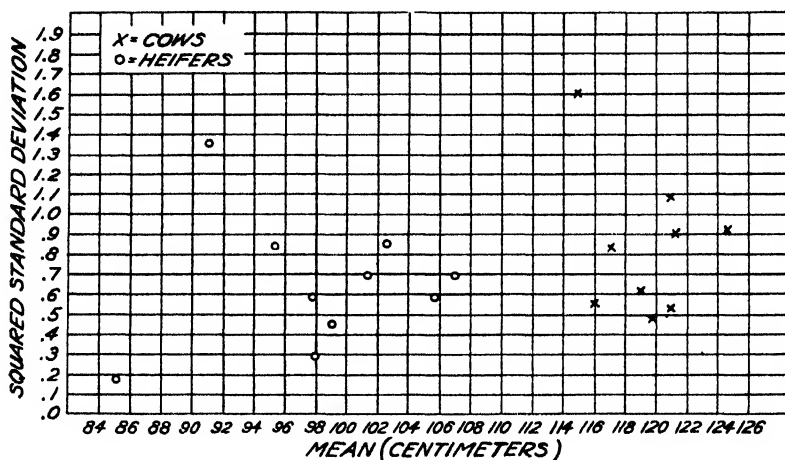


FIGURE 8.—Relation between observed standard deviation and mean size of measurements of height over withers for individual animals

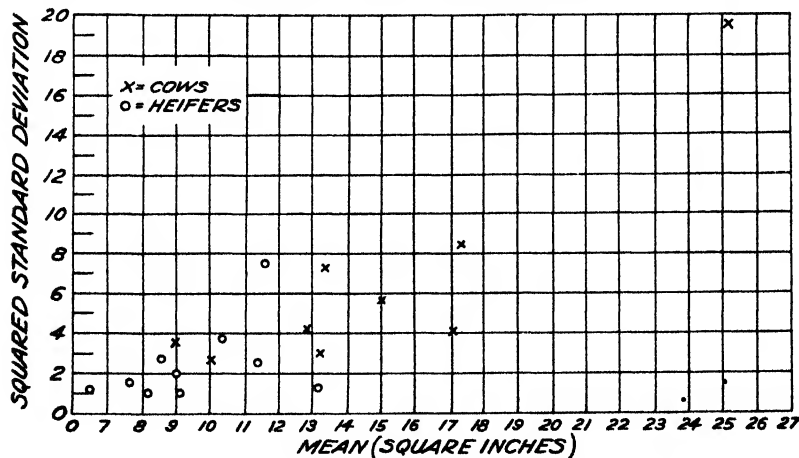


FIGURE 9.—Relation between observed standard deviation and mean size of measurements of dewlap area for individual animals

The figures in column 5 indicate that for these groups, single measurements were in most cases accurate enough to determine signifi-

⁸ Shewhart's formula is as follows:

$\sigma O = \sqrt{\sigma T^2 + \sigma E^2}$ where σO = the observed standard deviation
 σT = the true standard deviation free from errors of measurement
 σE = the standard deviation of the errors in measuring.

In the case of height at knee in these data this becomes

$$0.95 = \sqrt{\sigma T^2 + \frac{(.70)^2}{11}} \quad \text{whence } \sigma T = 0.92$$

cantly (in the statistical sense) which was the larger of two animals in the measurement concerned. Averages based on two or more repetitions of the measuring would of course be still more accurate. Thus Shewhart's formula becomes

$$\sigma O^2 = \sigma T^2 + \frac{\sigma E^2}{n}$$

where σO = the observed standard deviation

σT = the true standard deviation

σE = the standard deviation of the errors in measuring

and n = the number of repetitions of the measurement made on each animal.

As n is increased, σO approaches more closely to σT .

No amount of repetition of the measuring can eliminate entirely the error of measuring, but by using the figures in column 2 of Tables 3 and 4 one can make a rough correction for the influence of those errors on standard deviations and on coefficients of correlation calculated from the actual data.

Such corrections of course presuppose a random distribution of errors and are themselves subject to errors of sampling which make them only approximate on small populations. That is, there is no magic in these corrections which will rigidly eliminate the effect of errors in measurement. They do, however, provide an average correction for the bias toward large standard deviations and small correlations caused by errors of measurement.

GENERAL DISCUSSION

There is in general a close correspondence in the findings from the two groups of cattle, although the error was for most measurements distinctly larger for the larger animals. For the most part the measurements were found to be more accurate than the writers had expected. The accuracy of most of them compares rather favorably with the accuracy of weights where the standard error of weighing was found⁹ in most cases to be between 6 and 12 pounds, or not far from 1 per cent of the mean weight.

Most of the measurements seemed to be less affected by day-to-day changes in weather and other external conditions than weights were, but some, notably dewlap area, and perhaps also paunch girth and flank girth, were even more affected by such changes than weight was.

The principal objection to the extensive use of body measurements, at least with dairy cattle, seems to be not their inaccuracy but their inadequacy to describe the animal in a complete way. A very large number of measurements would be necessary to permit even a rough reconstruction of the surface shape of a cow, and the mere computation of such data for a group of animals is a formidable task. Moreover, the mental process of integrating a large number of such averages into a definite mental picture of an average animal would be exceedingly difficult, if possible at all. Perhaps it is theoretically possible that there is an equation which would represent a moving point tracing out the external form of a cow in the same way that the equa-

⁹ LUSH, J. L., CHRISTENSEN, F. W., WILSON, C. V., and BLACK, W. H. Op. cit.

tion $X^2 + Y^2 + Z^2 = A$ represents the surface of a sphere with the center at the origin. But such an equation would certainly have to contain hundreds of functions of X , Y , and Z , and the mere arithmetic of its computation would be too formidable a task even for the professional mathematician. Moreover, it would change at least slightly with every movement of the cow. And when one sought to compare or average two or more such equations still greater difficulties would be encountered. Such mathematically complete descriptions of the body shape of cattle seem clearly impossible.

The taking of more and more measurements in the interest of completeness (itself not achievable) involves the law of diminishing returns of new information for each additional unit of time and money spent in collecting and analyzing the data. For most purposes a very few measurements considered in relation to each other or in relation to weight seem as much as would be really useful in contributing to the general picture of the animal and of the changes which occurred in it. Thus height over withers or over hips would certainly be included among those measurements least influenced by plane of nutrition, and therefore especially apt to be illuminating when considered in relation to weight. Depth of chest, length of body, heart girth, paunch girth, and flank girth in relation to each other and to weight all give some information about the body. So do width at shoulders, width at loin, and width of chest, although these measurements are much affected by the degree of fatness. The investigation of certain ideas current among judges of livestock may warrant the special study of measurements of head and pelvis. But with completeness of description never attainable and with the labor of computation and analysis mounting more rapidly for each additional measurement, the investigation is apt soon to reach the point where the taking of additional measurements will lead to less rather than to more findings because of the increasing likelihood that the data will not be fully analyzed or that the investigator will use all his time and energy in the analysis of the measurement data, leaving untouched other more promising lines of attack.

SUMMARY

Twenty-five different measurements were each taken 11 times on each of 9 cows and 10 yearling heifers from the Jersey herd of the Texas Agricultural Experiment Station. From these data the usual error of measuring was calculated for each measurement of the cows and of the heifers. The two groups of data agree rather closely, although most of the measurements show somewhat larger errors for the cows than for the heifers.

In only a few measurements was the standard error of measuring much larger than 2 per cent of the measurement, and in about one-third of the measurements it was less than 1 per cent. The errors of measuring are of about the same magnitude as the errors of weighing when expressed as percentages of the mean.

There was a slight correlation between the mean size of a measurement for each animal and the standard deviation of the measurements taken on that animal. This varied for different measurements, and for some measurements would have been rather large if the data for the cows and the heifers had been included in a single population.

THE VITAMIN-C CONTENT OF COMMERCIALY CANNED SAUERKRAUT, TOGETHER WITH SOME OBSERVATIONS ON ITS VITAMIN-A CONTENT¹

By BERTHA CLOW, *Instructor*, HELEN T. PARSONS, *Associate Professor*, and INA STEVENSON, *Assistant Home Economist*, Department of Home Economics, University of Wisconsin²

INTRODUCTION

In a previous paper (1)³ it was reported that sauerkraut obtained directly from the barrel in which fermentation took place may contain approximately one-half as much vitamin C as that found in raw cabbage. This study seemed to establish the fact that there is an appreciable amount of vitamin C in sauerkraut as it comes from the fermentation vat. The purpose of the present investigation was to determine the vitamin-C content of sauerkraut as it reaches the consumer. In the course of these studies observations were made on the vitamin-A content, and these are also included.

EXPERIMENTAL MATERIAL

Sauerkraut put up in tin was obtained from six companies of the National Kraut Packers' Association. These companies were situated in Ohio, New York, and Wisconsin. At the beginning of the experiment in the latter part of January and the first of February, 1929, each company furnished three cases (24 cans per case) of sauerkraut together with the history of the shipment. From the data received it would seem that all but two brands were from the same lot; i. e., the date of "cutting" the cabbage and the date of canning the sauerkraut were uniform for that shipment.

The range of variation in some of the more important details in the manufacture of the sauerkraut studied is given in Table 1. This table shows a wide divergence in the processes used by the manufacturers of this product at the present time.

¹ Received for publication Dec. 7, 1929; issued June, 1930. Published with the permission of the director of the Wisconsin Agricultural Experiment Station. This study was made possible through an industrial fellowship fund given the University of Wisconsin by the National Kraut Packers' Association. The research carried out under the fund was planned and executed by members of the university staff and the work was done in the university laboratories. The participation of the National Kraut Packers' Association consisted in furnishing the funds for the payment of salaries, labor, and supplies and in contributing the samples of sauerkraut used in the tests.

² The authors wish to express their appreciation to Dr. A. L. Marlatt of the Department of Home Economics, to Dr. E. B. Fred of the Department of Agricultural Bacteriology, and to Dr. W. H. Peterson of the Department of Agricultural Chemistry for securing the cooperation of the National Kraut Packers' Association in this investigation.

³ Reference is made by number (italic) to "Literature cited," p. 64.

TABLE 1.—*Range of variation in some of the details of the processes of manufacture of the six brands of sauerkraut studied*

[These data were obtained from the various companies]

Item	Extremes of difference	
Length of time in the fermentation vat	124 days	Less than (0) days.
Extremes of temperature reported in the fermentation room	70° F.	24° F.
Extremes of temperature reported in the fermentation vat	77° F.	30° F.
Heating before packing		
Time	20 minutes	2 minutes.
Temperature	175° F.	85° F.
Method of packing	Machine	Hand
Heating before sealing the cans of the machine-packed sauerkraut		
Time	5 minutes	None.
Temperature	204° F.	Do
Heating before sealing the cans of the hand-packed sauerkraut		
Time	5 minutes	2 minutes
Temperature	210° F.	114° F.
Processing after sealing the cans of the machine-packed sauerkraut	None	None.
Processing after sealing the cans of the hand-packed sauerkraut		
Time	10 minutes	8 minutes.
Temperature	212° F.	180° F.
Acidity of sauerkraut		
Raw	1.90	1.58.
Canned	1.5	1.

* This information was not given by all 6 companies.

EXPERIMENTAL METHODS

VITAMIN C

The protection method was used in these studies. The criteria for adequate protection were satisfactory growth for 60 days and absence of scurvy symptoms during this time and at autopsy. Guinea pigs were used in the experiments, and for the most part they were obtained from commercial breeders. Young guinea pigs weighing about 200 gm. were put immediately on the basal ration plus cabbage, to which they had free access. From one to five days later, depending on the size of the animals, the experimental feeding was begun. The cabbage was ground in a food chopper, a small amount (5 to 10 gm.) of sauerkraut was added to the cabbage, and the mixture was put into the basal ration. Each day the amount of sauerkraut was increased, but at no time was the quantity of cabbage in the mixture less than 5 gm. per guinea pig in the group. The animals thus gradually became accustomed to the taste of the sauerkraut, and as a rule there was no trouble in getting them to eat it when the cabbage was withdrawn entirely. Except in a few cases, where the animals were large when received from the breeder, the guinea pigs weighed about 230 to 250 gm. when they were put in individual cages. The doses of sauerkraut were mixed only in the central portion of the ration and were usually consumed immediately. The basal ration consisted of the following: Alfalfa (autoclaved 30 minutes at 15 pounds pressure), 25 per cent; rolled oats, 69 per cent; purified casein, 5 per cent. The animals that were used as positive or negative controls were given the above ration with the addition of 1 per cent sodium chloride. The ration containing the salt is the one used by Ellis, Steenbock, and Hart (4). The casein was purified by soaking for a week in water slightly acidified with glacial acetic acid (approximately 5 c. c. per 6 quarts of tap water). This water was changed daily.

The first experiments were made with 7½ and 5 gm. daily doses, the idea being that after these animals had been on the experimental

ration long enough to give an indication of the results, the next group of guinea pigs could be started on 10 or $2\frac{1}{2}$ gm. doses. With each of the six brands of sauerkraut the $7\frac{1}{2}$ gm. dose proved to be within the protection level, and thus with each brand the degree of protection on $7\frac{1}{2}$, 5, and $2\frac{1}{2}$ gm. daily doses was determined.

In the experiments in which $7\frac{1}{2}$ and 5 gm. doses were used, fresh cans of sauerkraut were opened on Monday, Wednesday, Friday, and Saturday. The top layer was removed, ground in a food chopper, and the portions weighed out. A weighted dish which fitted into the can was put on the exposed layer of sauerkraut and pushed down so that the juice came up around it. These cans were then put into an electric refrigerator. On Tuesday and Thursday these opened cans were taken out of the refrigerator, and the top layer of sauerkraut and juice, amounting approximately to one-half the total contents of the can, was removed and discarded. The remaining sauerkraut was ground as on the previous day. The animals on the $7\frac{1}{2}$ and 5 gm. levels had single doses from Monday to Friday, inclusive, and double doses on Saturday. Those on the $2\frac{1}{2}$ -gm. level had double doses on Monday, Wednesday, and Saturday and single doses on Friday; thus they always received sauerkraut from a freshly opened can. Although the method of keeping the opened can of sauerkraut from one day until the next would seem to eliminate the factor of oxidation, it was desired that no question should arise as to oxidation when feeding at the lower level of $2\frac{1}{2}$ gm.

Before each day's dose was given the food remaining in the dish from the day before was put through a sieve that allowed the basal ration to pass through but retained the sauerkraut if any were present. It was not possible to make a quantitative estimate of the amount of sauerkraut thus remaining, but all animals which after a short period of trial failed to eat the entire dose were either discarded or shifted to a lower level.

The criteria for determining the appearance of scurvy in the guinea pigs and the degree of its severity were: Stiffness of the joints and a characteristic uneven gait in running; wincing and crying on being handled; a swelling of the wrists and arms; an awkward position of the knees held away from contact with the body; and, occasionally, the "face-ache" position. The criteria on autopsy were swelling of the arms and legs; hemorrhage of the joints, muscles, and skin; looseness or brittleness of the teeth; brittleness of the leg bones; cloudiness, hemorrhage, and beading of the ribs.

VITAMIN A

Young rats from 21 to 28 days of age weighing 40 to 45 gm. were placed on the following vitamin A low ration: Purified casein, 18 per cent; salt mixture (Osborne and Mendel), 4 per cent; agar, 2 per cent; dried brewer's yeast (irradiated), 8 per cent; cooked cornstarch, 68 per cent. The ration was similar to that of Steenbock and Coward (9) except for the treatment of the starch and the yeast and the substitution of another salt mixture (7) which was being used at the time in other rations in the laboratory, and was also used in Sherman's vitamin A low ration (8). The casein was extracted with hot 95 per cent alcohol for approximately 50 hours and then heated at 98° C. for a week in a drying oven. It assumed a decidedly brown tinge during the process without, however, losing its palatability. The

dried commercial brewer's yeast was irradiated under a quartz mercury vapor lamp for 10 minutes at a distance of 18 inches in order to provide a convenient and adequate source of vitamin D. The starch was cooked in boiling distilled water until translucent and was then dried and ground.

The animals were caged on wire screens in groups during the depletion period, but separately at the first symptom of eye disease. The criterion for judging the depletion of vitamin A in the rats was in all cases the appearance of pronounced sore eyes and not primarily the cessation of growth. The criteria for the response of the animal to the presence of vitamin A in the doses were both the rate of growth and the speed and completeness of the recovery from sore eyes.

The doses of sauerkraut were prepared like those for the vitamin-C determinations and were fed separately in glass casserole cups placed in larger metal cups to prevent spilling. Because of the difficulty which was encountered in securing quantitative intake of the sauerkraut it was found necessary in a good many instances to collect the uneaten portion of the dose, dry it to constant weight, and calculate from determinations of the dry matter of the sauerkraut what part of the dose had actually been eaten. Doses of cabbage were prepared by removing damaged or soiled leaves from a head of cabbage and taking a slice through the head to include representative portions of both the white inner and the greenish outer leaves. Doses were weighed out from the shredded pieces of this slice.

For reasons that will later be explained only one brand of sauerkraut was used for vitamin-A determinations. Brand C was selected because a sample of this was the first to arrive in the laboratory and was ready for feeding when the animals were depleted of their vitamin A stores.

PRESENTATION OF RESULTS

The six brands of sauerkraut studied are indicated by letter and will be discussed in the order of their effectiveness as antiscorbutic substances, the most effective coming first. At least four guinea pigs and in most cases five were started on each level on each brand of sauerkraut. In one instance as many as 16 were started on a single dose. The data presented in this paper include all animals except those which were discarded for the following reasons: Failure to eat sauerkraut; refusal to eat the basal ration with resultant starvation; and the presence of complicating factors such as infected lungs or liver. Such infections could not be determined until autopsy was performed, and in several instances this was at the end of the experimental period or so near the end that there was not time enough to start other animals to replace those discarded. In the case of animals that refused to eat sauerkraut or the basal ration, it was possible to substitute others and thus keep the number in each group to at least four. In only three groups—those fed 5 gm. of brand A and 2½ gm. of brands D and E—are there less than four animals included in the final report. The total number of guinea pigs started on sauerkraut doses was 110, while the number included in this report is 80. Thus there were 30 animals started on doses which it was necessary to discard.

VITAMIN C

CONTROL EXPERIMENT (FIG. 1)

By reference to Figure 1 it will be observed that the negative control animals, which received only the basal ration, developed scurvy in 15 to 20 days. Animals Nos. 25 and 26 were fed at the beginning of the studies and Nos. 146, 147, and 148 toward the end. There was no change in the source of the supplies for the ration.

With the addition of a known source of vitamin C the basal ration is shown to be adequate for growth. Five grams of cabbage is prob-

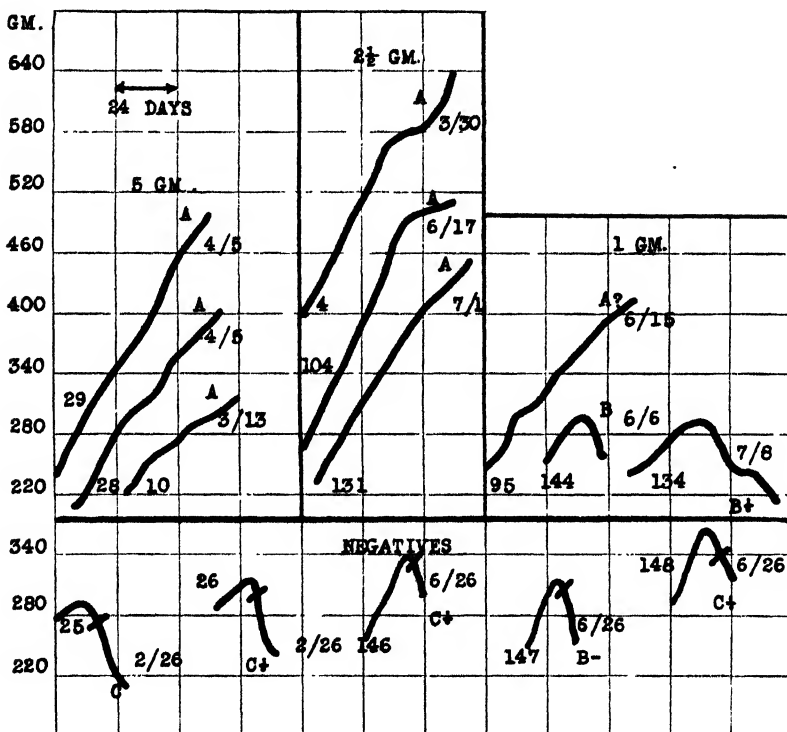


FIGURE 1.—Control experiments on vitamin C. The positive control animals received 5, 2½, and 1 gm. doses of raw cabbage daily. Animals 28 and 29 had, in addition to cabbage, varying amounts of sauerkraut chosen at random. Negative control animals were on the basal ration alone; cross lines on their weight curves indicate the point at which scurvy became evident. In all figures the numbers near autopsy indicate the month and day of the month on which autopsy was performed; autopsy symbols used as follows: A, No sign of scurvy; A?, possibility of scurvy; A—, slight but definite signs of scurvy; B+, moderate scurvy; B, B—, C+, and C, increasing degrees of scurvy

ably a very high protection level, although animal 10 did not seem to respond to the addition of this quantity with very good growth. Animals 28 and 29 were given the cabbage and sauerkraut mixture such as all the animals received during the preliminary feeding period, that is, 5 gm. of cabbage per guinea pig plus various amounts of different brands of sauerkraut chosen at random. This was done to make sure that the practice of mixing sauerkraut with the cabbage to accustom the animals to the flavor would not produce an undesirable

effect during the period before the experiment was begun. Both animals showed excellent growth and no signs of scurvy.

The 2½-gm. level of raw cabbage seems to give good growth and complete protection from scurvy. As was pointed out in a previous paper (1) this quantity of cabbage gave good growth with two animals for 44 to 58 days, but after that time there was a "flattening off" of the weight curve. In the present studies one animal, No. 104, shows a flattened curve for the last 18 days of the 60-day experimental period, while the other two animals show consistently good growth throughout the 60 days.

The 1-gm. level of feeding raw cabbage does not seem to be adequate for complete protection, although in one instance there was good

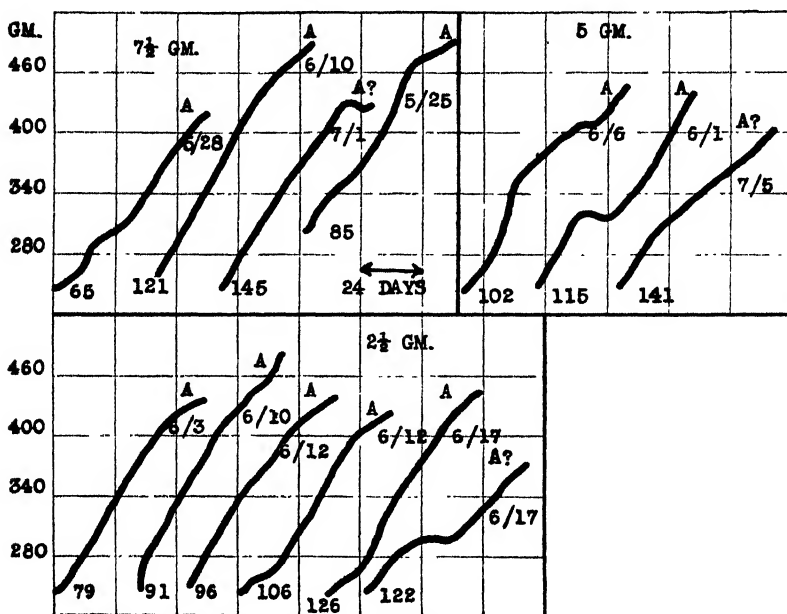


FIGURE 2—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand A. For explanation of symbols see Figure 1

growth, i. e., animal 95. (Fig. 1.) Animal 144 was chloroformed at the end of 24 days and at autopsy showed definite signs of scurvy. Animal 134, also receiving 1 gm. of cabbage, lived throughout the 60-day experimental period but showed a decline in weight after 30 days and definite symptoms of scurvy at autopsy. (Fig. 1.) It thus appears that 1 gm. of raw cabbage is the smallest quantity that will give any degree of protection.

SAUERKRAUT, BRAND A (FIG. 2)

Of all the six brands studied by far the best results were obtained with brand A. The average daily gain in weight of the four animals on the 7½-gm. level was 3.28 gm.; those on the 5-gm. level, 3.2 gm.; and those on the 2½-gm. level, 3.11 gm. Thus, as far as growth is concerned, the lowest level seems to be practically as effective as the highest. The protection from scurvy at each of the three levels is

also very good. In only three cases was there a question at autopsy as to whether complete protection had been afforded, namely, animals 145, 141, and 122 receiving $7\frac{1}{2}$, 5, and $2\frac{1}{2}$ gm., respectively. It will be noted that the growth of these animals was not so good as that of the others receiving the same dose of this brand of sauerkraut. It should also be noted (see autopsy dates on curves, fig. 2) that autopsies were performed on guinea pig 145 (receiving $7\frac{1}{2}$ gm.) and 141 (receiving 5 gm.) three weeks or more after the other animals in the group. A new case of 24 cans of sauerkraut was opened on June 14. It is possible that the last case of material was not so good as the others, or it may have been that warm weather during the latter part of June effected a partial destruction of vitamin C in the sauerkraut both before and after the cans were opened. However, the variation in temperature in both the storage rooms and the animal laboratory

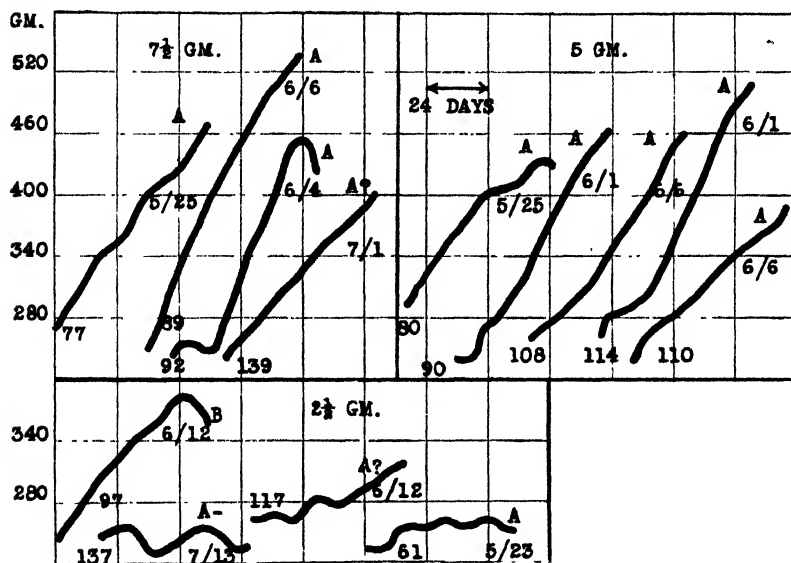


FIGURE 3. -Growth of guinea pigs and degree of protection from scurvy afforded them by $7\frac{1}{2}$, 5, and $2\frac{1}{2}$ gm. daily doses of commercial sauerkraut, brand B. For explanation of symbols see Figure 1.

was only 4° to 5° F. Further than the points made above there seems to be no obvious explanation for a condition of scurvy on the $7\frac{1}{2}$ and 5 gm. doses of sauerkraut A. It seems rather significant, however, that five out of six animals receiving $2\frac{1}{2}$ gm. of brand A show complete protection.

SAUERKRAUT, BRAND B (FIG. 3)

With only one exception (animal 139) the $7\frac{1}{2}$ and 5 gm. levels seemed to give protection from scurvy and also very good growth, the average daily gain in weight for the group being 3.41 and 3.23 gm., respectively. Guinea pig 139, receiving $7\frac{1}{2}$ gm., was probably not completely protected from scurvy. As in the case of the two animals on brand A (Nos. 145 and 141), the 60-day experimental period extended more than three weeks after that of any other animal in the group; also a new case of sauerkraut was opened on June 14. The

2½-gm. level seems to be what might be termed "the border-line dose." On such a dose the results appear to be inconsistent; for example, in this case, one animal (No. 61) maintained its weight throughout the 60 days, and on autopsy was found to be free from symptoms of scurvy; another animal (No. 97) grew very well during most of the experimental period, but autopsy showed a moderate condition of scurvy. The other two animals gave results intermediate between these. It should be noted that although there was evidence

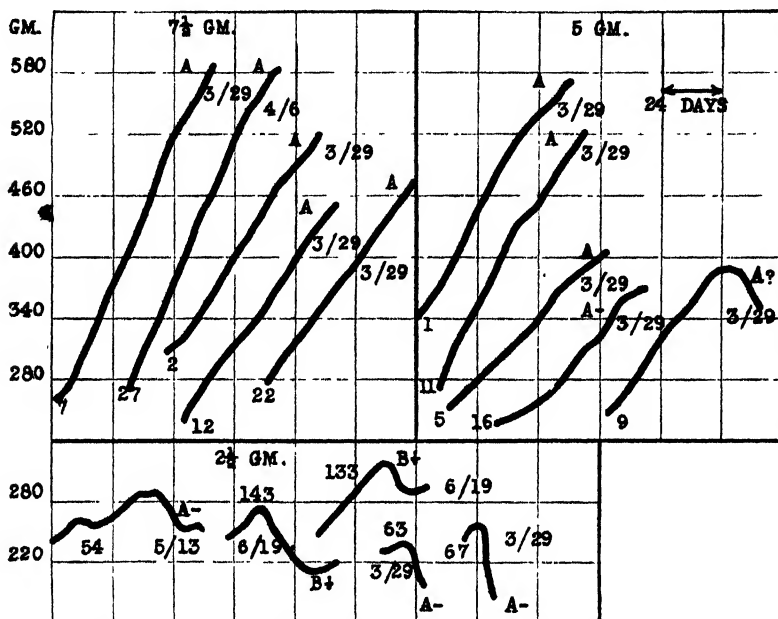


FIGURE 4.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand C. For explanation of symbols see Figure 1

of scurvy in some cases all animals lived throughout the 60-day experimental period.

SAUERKRAUT, BRAND C (FIG. 4)

The 7½-gm. level seemed to give complete protection from scurvy and also very good growth, the average daily gain in weight for the group being 4.39 gm. The 5-gm. level, although giving an average daily gain of 3.23 gm. for the group, did not give complete protection from scurvy in every case. Guinea pig 16 had a definite although slight hemorrhage on each of the hind legs. Guinea pig 9 seemed to have slightly brittle bones. Thus it can not be said that the 5-gm. level of brand C gives complete protection, but because of the fairly consistent good growth this level should doubtless be considered slightly above the "border-line" dose. The 2½-gm. level is quite definitely below the border-line dose, as in each case there was evidence of scurvy and only one animal lived 60 days.

SAUERKRAUT, BRAND D (FIG. 5)

The 7½-gm. level seems to give complete protection from scurvy and also good growth, the average daily gain in weight for the group being 3.2 gm. The 5-gm. level can probably be considered the border-line dose, as in three out of four cases there were symptoms of scurvy and two of the animals did not survive the 60-day experimental period. Although growth was good in two cases and there was complete protection in one case (No. 70), the evidence indicates that a 5-gm. level of brand D is at least very close to the border-line dose. The 2½-gm. level gave evidence of scurvy in every case, fair growth in

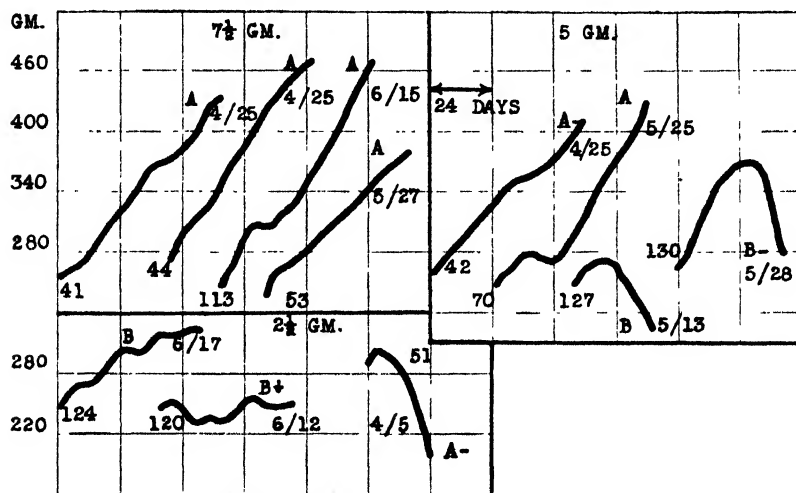


FIGURE 5—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand D. For explanation of symbols see Figure 1

one case (No. 124), and the 60-day survival period in two cases (Nos. 120 and 124).

SAUERKRAUT, BRAND E (FIG. 6)

The 7½-gm. level seems to give complete protection from scurvy and also good growth, the average daily gain in weight for the group being 3.63 gm. The 5-gm. level seemed to give good growth in four cases and fair growth in one case, the average daily gain in weight for the group being 2.96 gm. The protection from scurvy seemed to be complete in the case of two animals, while the other three on the 5-gm. level showed evidence of scurvy. The four animals on the 2½-gm. level all had scurvy, and the survival period was very short, showing that this dosage is below the protection level.

SAUERKRAUT, BRAND F (FIG. 7)

In three cases the 7½-gm. level seemed to give complete protection and also good growth, the average daily gain in weight for the animals on this dose being 3.62 gm. The fourth animal in the group (No. 135) gained weight for 36 days and then lost weight so rapidly that it was chloroformed at the end of 50 days, the autopsy showing slight but positive signs of scurvy. The 5-gm. level can probably

be considered the border-line dose, as in only two cases out of seven was there complete protection. Growth was neither consistently good nor poor. The 2½-gm. level was doubtless below the protection dose as in every case the animal had scurvy and the survival period was short, the longest being 37 days in the case of animal 58.

Another factor must be considered in evaluating sauerkraut F. The total number of animals started on doses of this brand was 28, whereas the highest total on any other brand was 19. There seemed to be difficulty in bringing the animals through a satisfactory experimental period. They did not refuse the sauerkraut, but ate very little of the basal ration after about the first 10 to 15 days, and death followed about 10 or 15 days later. Autopsy showed no scurvy, but there was usually a good deal of gas in the intestinal tract. Most

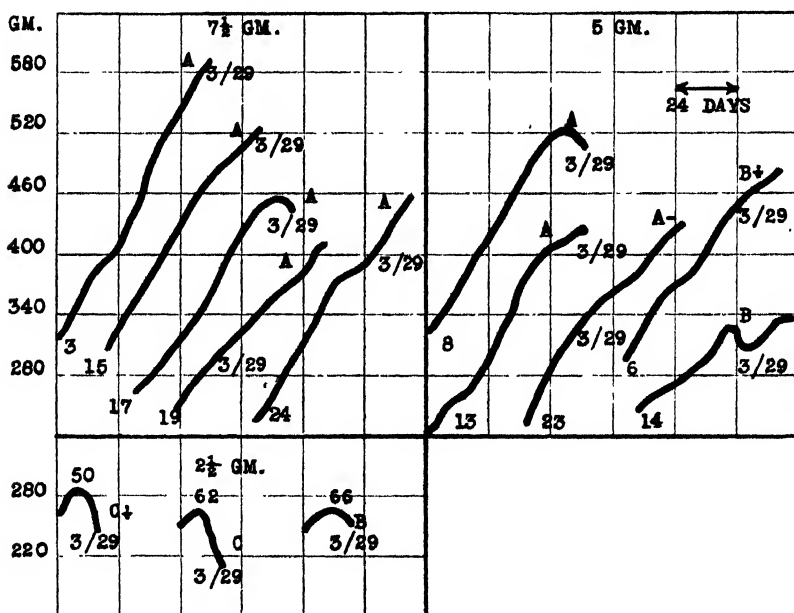


FIGURE 6—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand E. For explanation of symbols see Figure 1

of these animals were on the 5-gm. dose; in fact the failures on this level were so numerous that 16 animals were used. The writers are unable to explain these failures. Such results were not obtained with any of the other brands tested.

COMPARISON OF BRANDS A TO F WITH FRESH RAW SAUERKRAUT

As pointed out in the introduction, the previous work (1) on raw sauerkraut obtained directly from the barrel in which fermentation took place showed that 5 gm. gave complete protection, while 2½ gm. was considered the minimum protection level, i. e., the border-line dose. Two brands, namely, A and B, compare very favorably with this raw sauerkraut. Commercial brands C, D, and E are only fairly comparable, while F is probably about one-half as effective as the raw sauerkraut.

VITAMIN A

In Table 2 is presented a record of the daily doses of sauerkraut consumed by the experimental rats in addition to the basal vitamin A low ration after symptoms of depletion of vitamin A developed. As may be seen from the table, quantitative consumption of a fixed dose could be secured for only a part of the 8-week period in any of the animals. Food intake records showed that the failure of the animals to eat greater quantities of the sauerkraut could not be

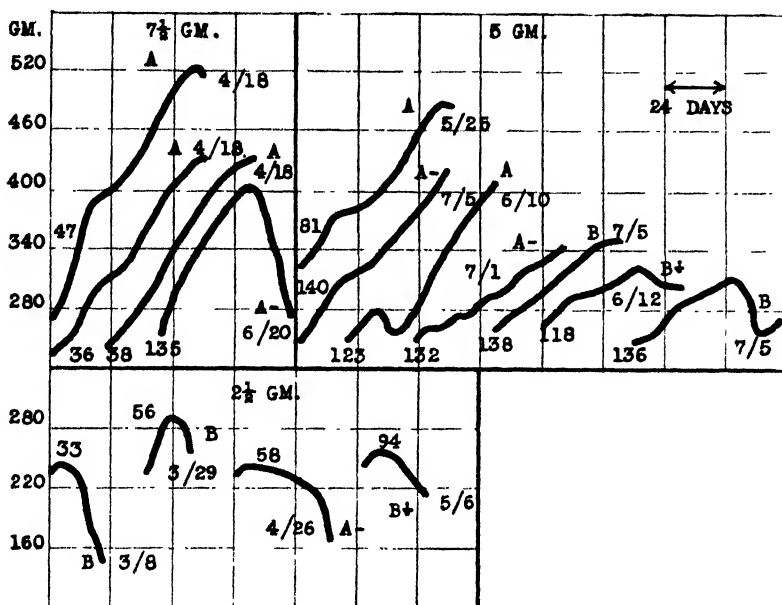


FIGURE 7.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½, 5, and 2½ gm. daily doses of commercial sauerkraut, brand F. For explanation of symbols see Figure 1

attributed to lack of appetite induced by too low a consumption of vitamin B complex in the basal ration.

TABLE 2.—Doses of sauerkraut consumed by rats following depletion of a vitamin A low diet

[Calculations are for a preliminary period when dosage was being established, for a period when quantitative intake of fixed doses was established; for a later period when intake again fell off; and for the total period of feeding]

Rat No.	Average daily dose for preliminary period 1		Length of period	Uniform daily dose in period 2		Length of period	Average daily dose for subsequent period 3		Length of period	Average daily dose for entire period of feeding	
	Grams	Days		Grams	Days		Grams	Days		Grams	Days
16	5.4	8		7	8		4.7	41		5.0	
28	4.1	18		5	20		4.2	23		4.5	
20	3.5	18		5	24		2.6	19		4.1	
30				(*)						3.5	
32	4.2	8		6	47		4.5	6		5.6	
35	2.8	10		5	15		3.4	36		3.7	
36	6.5	2		12	10		5	43		6.7	
39	2.8	6		4	3		2	43		2.1	

* This rat did not at any time consume the whole of the dose weighed out.

In view of this irregularity in dosage, therefore, only a general comparison can be made between the vitamin-A content of the canned sauerkraut and that of the cabbage. The results in Figures 8 and 9 show that a daily intake of 3 to 4 gm. of stored winter cabbage leads to approximately the same gain in weight in depleted rats as that induced by an average daily intake of 4 to 6.7 gm. of

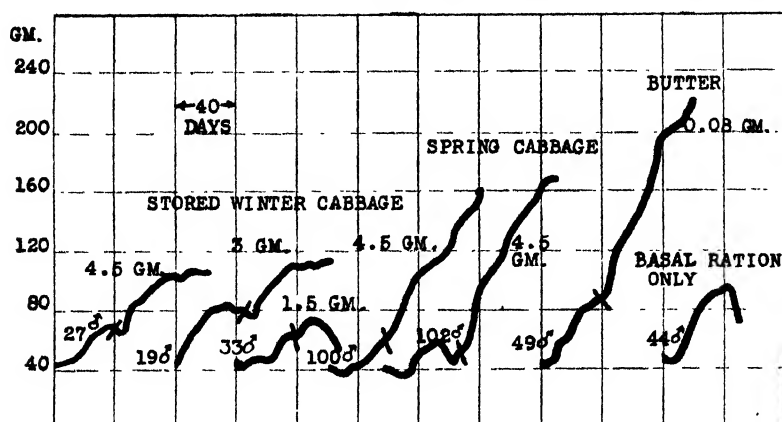


FIGURE 8.—Control experiments on vitamin A. Growth of rats during a depletion period on a vitamin-A low ration and also during a period when weighed doses of stored winter cabbage, spring cabbage, or butter were fed. Cross lines on the curves indicate the beginning of dosages. In one negative experiment the supplementary doses were not fed. For explanation of symbols see Figure 1

the canned sauerkraut. These levels are also comparable in their effect on sore eyes. This brand of sauerkraut therefore contains, roughly, one-half to three-fourths as much vitamin A as does stored winter cabbage of the type used in the manufacture of sauerkraut. Spring cabbage fed to rats 100 and 102 after the winter variety fed to the other rats was unobtainable proved to be a richer source

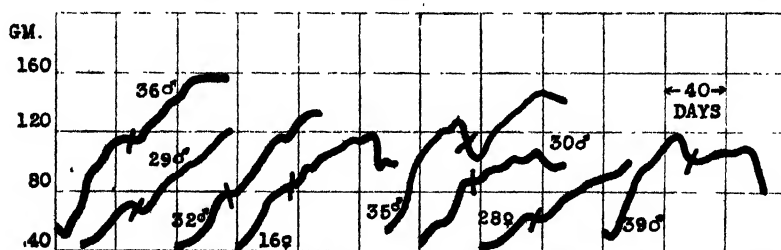


FIGURE 9.—Growth of rats during a preliminary depletion period on a vitamin-A low ration and also during a period when supplementary weighed doses of commercial sauerkraut of brand C were fed. Cross lines on the curves indicate the beginning of dosages. The intake of sauerkraut was so irregular that reference must be made to Table 2 for the size of doses consumed. For explanation of symbol see Figure 1

of vitamin A than winter cabbage, as would be expected from its smaller open head and greener color. A wide difference in the nutritive value of white and green leaves of cabbage has been noted by Hume (5), Delf (3), Coward and Drummond (2), and Steenbock and Sell (10). The practice among manufacturers of sauerkraut varies from removing only the damaged or soiled outer leaves to stripping the head until it is "white." Le Fevre (6, p. 9) states

"Sauerkraut made from green cabbage will show defects in color and texture." It is possible, therefore, that the difference in vitamin-A content noted between this brand of canned sauerkraut and the stored winter cabbage tested may be attributable largely to a difference in their content of vitamin A after stripping rather than to losses during the fermentation and canning of the sauerkraut. It should be clearly recognized, however, that the amount of vitamin A in winter cabbage is practically negligible as a source of vitamin A in the diet. For example, it would require approximately 20 to 25 pounds of the winter cabbage used in this experiment to furnish as much vitamin A as would be furnished by 1 pound of the butter used. It seems probable from the experiment that 30 to 45 pounds of the brand C canned sauerkraut studied would be needed to furnish this same amount of vitamin A.

SUMMARY

VITAMIN C

A study of the vitamin-C content of six brands of commercially canned sauerkraut has been made. The protection method was used, with guinea pigs as the experimental animals. Levels of $7\frac{1}{2}$, 5, and $2\frac{1}{2}$ gm. were employed. There was found to be considerable variation in the vitamin-C content of the six brands tested. Brand A was outstanding because of the good growth and protection it afforded on the $2\frac{1}{2}$ -gm. level. Brand B gave good growth and protection on the $7\frac{1}{2}$ and 5 gm. levels but definite evidence of scurvy on the $2\frac{1}{2}$ -gm. level. Brand C, D, and E gave evidence of scurvy on the 5-gm. level. Brand F not only gave evidence of scurvy on the 5-gm. level but also on the $7\frac{1}{2}$ -gm. level. Furthermore, with brand F there was difficulty in bringing the animals through a satisfactory experimental period.

The vitamin-C content of brands A and B compare very favorably with that of specially fermented fresh raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin (*1*). C, D, and E are distinctly less effective than the raw sauerkraut, while F probably contains about one-half the amount of vitamin C found in the raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin.

Because of the lack of uniformity in the methods used in the manufacture of the sauerkraut tested, it was not possible to determine what factor or set of factors was responsible for the loss of vitamin C in the poorer brands.

No conclusions as to the probable vitamin-C content of products of the sauerkraut industry other than the sauerkraut itself put up in tin containers are warranted by these experiments.

VITAMIN A

A study of the vitamin-A content of one brand of commercially canned sauerkraut has been made with the rat as the experimental animal. The method used included the depletion of the animal's body stores of vitamin A until sore eyes appeared, and a subsequent supplying of daily weighed doses of the material to be tested in an attempt to cure sore eyes and promote normal growth. In attempting to study the comparative vitamin-A content of cabbage and

sauerkraut two difficulties in particular were encountered. Quantitative intake of doses of sauerkraut could be secured over only a part of the desired time with any of the rats, and this at levels too low to permit complete cure of the sore eyes produced during the depletion period. Difficulty was also encountered in securing satisfactory control experiments because of the unequal distribution of vitamin A in cabbage. These two limitations have made it impossible to arrive at any accurate determination of the possible losses of vitamin A during the processes of manufacture and canning of commercial sauerkraut. The results of the study of one brand suggest that differences between the vitamin-A content of sauerkraut and cabbage may be due to variations in the type of cabbage used or to the extent of the removal of the outer leaves of the head more largely than to any considerable destruction of vitamin A during the processes of fermentation and canning. The amount of vitamin A contributed to the diet by either sauerkraut or winter cabbage is practically negligible.

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POTASSIUM IODIDE AS A MINERAL SUPPLEMENT IN PAIRED FEEDING EXPERIMENTS WITH GROWING SWINE¹

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INTRODUCTION

During recent years the advisability of reinforcing the rations of farm animals with additional minerals has received a great deal of investigation by a number of agricultural experiment stations in this country. The mineral supplements investigated, both singly and in various combinations, have been numerous. From the mass of experimental data obtained certain facts have been established beyond question. With reference to the feeding of swine, the prevalence of a calcium deficiency in the grains and their by-products has been demonstrated, and methods of correcting this deficiency, either with supplementary feeds or with mineral compounds of calcium, have been worked out. The value of salt in swine rations has been repeatedly confirmed, and the need for iodine supplements by pregnant sows in certain restricted areas has been clearly shown. Beyond these basic facts, more or less confusion prevails as regards the need for other minerals, a confusion of which commercial interests have taken the fullest advantage.

It appeared to the writers of this paper that much of this confusion about the effects of adding mineral supplements to swine rations was because of the method of experimental feeding employed. The results of the group-feeding method, particularly when no attempt is made to equalize the intake of food, are not so readily and surely interpreted as is commonly supposed, and their full explanation is in most cases impossible.

REVIEW OF LITERATURE

The situation can be well illustrated with reference to the mineral supplement with which this paper is specifically concerned, i. e., potassium iodide. An exhaustive review of the published experimental work on Iodine in Nutrition, prepared recently by Orr and Leitch (10),² removes the necessity of any extensive review of this work here. The following discussion is not concerned with the need of iodine by pregnant sows in those areas within which goiter is endemic, thus restricting the field of experimental work to which specific attention should be directed. It deals only with the possible need for iodine supplements by young growing swine in those areas of the country, including Illinois, in which goiter in farm animals is either nonexistent or of rare occurrence. Welch (15), as the result

¹ Received for publication Feb. 1, 1930; issued, June, 1930.

² Reference is made by number (italics) to "Literature cited," p. 77.

of a study of goiter in farm animals that extended over a number of years, came to the conclusion that the geographic distribution of animal goiter in this country is much more restricted than that of human goiter.

In 1925 Evvard and Culbertson (5) published the results of three experiments on young growing pigs concerned with the effect on growth of a potassium iodide supplement to good rations. In the first experiment the pigs were fed a basal ration of corn with a supplemental mixture containing tankage, corn gluten meal, corn oil cake meal, linseed meal, cottonseed meal, bone meal, and salt. They had access to rape pasture and additional salt. The second and third experiments involved dry-lot feeding. The basal ration in the second experiment contained corn, cottonseed meal, blood meal, limestone, salt, and bone meal; and in the third experiment it was more complicated, containing besides corn, cottonseed meal, corn oil cake meal, linseed meal, standard wheat middlings, soybean meal, peanut meal, alfalfa meal, salt, limestone, and spent bone black. In the iodide-fed lots a small amount of potassium iodide was introduced either into the supplemental feed or the supplemental minerals. The average daily intakes of potassium iodide in the three experiments were, respectively, 0.67, 0.85, and 0.08 grain per pig, or 43, 55, and 5 mgm. The experimental lots contained, respectively, 5, 7, and 6 pigs each in the three experiments.

In all experiments the rate and economy of gain averaged higher in the iodide-fed than in the control lot, and in the last two experiments the iodide-fed pigs averaged larger in length, height, and circumference. The significance of the experiment is assessed, by the authors and by those referring to it, largely on the basis of the average results only, and the inference is that the iodide supplement increased the rate of gain and decreased the cost by approximately 10 per cent. However, the probable errors of the average daily gains have been computed, and the average difference between comparable lots with their probable errors are reported for all experiments. These are, respectively, 0.103 ± 0.091 , 0.126 ± 0.092 , and 0.179 ± 0.052 pound. This statistical analysis affords no basis for supposing that, in the first two experiments, the greater average gain of the iodide-fed lot was the result of iodide feeding, since the average difference in gain is only slightly greater than its probable error.

The interpretation of these differences and their probable errors by the authors (5, *p. 195*) of the report may be illustrated by the statement in regard to the first experiment:

The mean difference is therefore 1.14 times the probable error. This would lead us to expect that future experiments, similarly planned and conducted, would result in approximately 78 per cent of the cases showing that the addition of the iodide would result in an increased average gain.

Serious exception may be taken to such an interpretation. This method of analysis simply permits an estimation of the probability that the particular outcome is the result of chance only. If this probability is sufficiently small, the operation of the imposed experimental condition becomes highly probable, or even practically certain. Anything greater than such a limiting probability, which is generally taken as 1 to 30 or 1 to 50, is commonly interpreted as a negative result. The particular experiment in question is undoubt-

edly of this description. The authors (5, p. 215) attach considerable significance to the fact that in all three experiments the variability of the gains in the iodide-fed lot was less than that in the control lot. These facts, according to the authors, "make the beneficial results secured from iodide feeding, under Ames conditions, quite significant." The basis of this argument is not apparent.

The usual interpretation of the third experiment would be that the rates of gain of the pigs had been favorably affected by the iodide supplement. However, as Student (1) showed in his paper of 1908, with small samples of 10 or less the standard deviation of the population can only be roughly estimated from that of the sample, and the errors of estimation seriously affect the use of the probable error, so that greater confidence in the significance of the experimental outcome is indicated than a more critical analysis would justify. Hence, in regard to the third experiment of Evvard and Culbertson (5), some hesitation in accepting the usual interpretation may reasonably be urged, since there were only six pigs to the lot. It may also be pertinent to call attention to the fact that the iodide dosage in the third experiment was only one-eighth that of the first experiment and one-eleventh that of the second. As the latter doses are not at all excessive or detrimental in their physiological effect, some surprise may be felt that the dose which was by far the smallest should produce the greatest effect.

A similar analysis of the dimensional measurements is not given by Evvard and Culbertson (5) and can not be made by the reader since the individual data are not at hand. Also, in group-feeding work, the statistical significance of average values for the economy of gains can not be assessed. It is not surprising, however, that lots showing the greater average rate of growth should also show the greater average size, as well as the greater average economy of gain. These are correlated, not independent, measurements.

In the course of a series of investigations of the mineral and vitamin requirements of pigs, Bohstedt and his associates (2), in their eighth experiment, tested the effect of an addition of potassium iodide to a ration of corn, flour wheat middlings, linseed meal, salt, calcium carbonate, and ferric oxide. Two groups of five young pigs each were used for this comparison. The control lot gained an average of 0.93 pound daily, and the iodide-fed lot, 1.01 pounds. The authors' statement that the iodide supplement seemed to improve the basal ration is based upon average group results, apparently without reference to individual performance.³ Unfortunately this is the usual method of interpreting group-feeding data, though it is clearly at fault. In this experiment also, no reference is made to the average intake of food. The control pigs consumed daily an average of 4.25 pounds of the ration, while the iodide-fed pigs consumed 4.8 pounds of feed daily. Hence, the control lot did not really function as a control lot should, since it affords no information concerning the gain that would have resulted from a daily intake of 4.8 pounds of the basal ration. The lack of such information makes it impossible to

³ The individual growth curves of the pigs in the iodide-fed lot and its control (2, p. 214-215) show clearly that the greater average gain of the former lot was due to the exceptionally rapid growth of two of the five pigs in the lot.

decide how much, if any, of the average difference in rate of gain between the two lots was caused by the potassium-iodide supplement.

In 1927, Weiser and Zaitschek (14) of Budapest reported an experiment on swine concerned with the influence of potassium iodide administered to the dam upon the growth and health of the pigs throughout the suckling period. Forty pregnant sows were divided into two groups, the first containing 17, and the second 23 animals. The feeding and treatment of the animals in the two groups were the same except that the sows of Group 2 received daily, during the last three weeks of pregnancy and throughout the lactation period, 125 mgm. of potassium iodide. In the aggregate, the results are described as follows:

The litters in the two groups averaged initially very nearly the same in size and weight. Slightly more of the pigs in the iodide-fed group were born dead, and considerably more were crushed by the mother sow. The striking differences between the two groups to which the authors call particular attention relate to the mortality incurred and to the growth secured. During the period of observation, almost 55 per cent of the pigs in Group 1 died of disease, the nature of which was not determined. In the same period, less than 3 per cent of the pigs in Group 2 succumbed. The average growth rate of the pigs in the latter group is said to have exceeded that of the former by a considerable amount, so that at the end of the experiment the iodide-fed pigs averaged 18.54 kgm. in weight, while the control pigs averaged only 13.17 kgm.

The experiment is thus presented as a clear-cut demonstration of a markedly favorable effect of iodide feeding on growth and resistance to disease. However, there are serious objections to the manner in which the experiments were carried out. All of the litters in Group 1 were farrowed during the period from November 13 to December 23, and all of those in Group 2 were farrowed during the period from November 5 to November 14. Thus, the two groups were not under observation simultaneously, and all of the litters of Group 2 were older than the litters of Group 1 with two exceptions. The weights obtained at any definite time are not comparable, though they are treated as such with no reference to the difference in age. Thus, the average final weights already cited refer in Group 1, to litters ranging from 60 to 75 days in age, averaging 66 days, and in Group 2, to litters ranging from 74 to 83 days in age, averaging 78 days. The average weights of pigs for the surviving litters when arranged in order of their ages present a regularly descending series of values showing no break in passing from the iodide-fed to the control group, as shown in Table 1. Obviously any conclusion that the administration of potassium iodide to the sows of Group 2 affected in any way the growth of the litters is untenable. The authors of this report show an amazing disregard of the obvious explanation of the differences in weight existing among the iodide-fed and control litters, and permitted an amazing delinquency in the planning of their experiment.

TABLE 1.—Average final weights of pigs per litter in the experiment of Weiser and Zaitschek (14), in comparison with age and iodine treatment

Sow No.	Age of litter	Average weight per pig	Group	Sow No.	Age of litter	Average weight per pig	Group
	<i>Days</i>	<i>Kgm.</i>			<i>Days</i>	<i>Kgm.</i>	
1	83	12.00	Iodide.	18	76	18.88	Iodide
2	83	19.50	Do	19	75	15.67	Do.
3	82	20.25	Do	1	75	15.71	Control.
4	82	21.80	Do.	2	75	9.00	Do.
5	81	18.88	Do	20	74	14.88	Iodide.
6	81	21.00	Do	21	74	14.33	Do.
7	80	18.29	Do.	22	74	18.30	Do
8	80	17.88	Do.	23	74	14.88	Do
9	79	16.33	Do	3	70	15.67	Control.
10	78	17.67	Do	4	70	10.86	Do
11	78	25.17	Do	5	63	15.00	Do
12	78	17.13	Do	6	63	12.00	Do.
13	78	18.82	Do.	7	61	14.86	Do.
14	77	18.78	Do.	8	60	13.83	Do
15	77	19.00	Do.	9	60	14.00	Do
16	76	22.67	Do.	10	60	10.00	Do.
17	76	19.14	Do.				

Consideration of the question whether the iodide administered to the sows of Group 2 protected their litters against the disease that decimated the litters in Group 1, must give due weight to the fact that over 80 per cent of the mortality in Group 1 occurred in 7 of the 17 litters, and that these 7 litters were the latest to be farrowed in that group. The 7 litters farrowed on and after December 2, containing 68 pigs at birth, were entirely destroyed before the final date of the experiment; the causes of death were not noted. Although there would be little profit in speculating on this matter, it is clear that conditions prevailing in December were very unfavorable to the growth and health of newborn pigs. Whether the supplemental iodide given the sows in Group 2 would have protected their pigs against these unfavorable conditions at a younger age can hardly be predicted from the outcome of this poorly planned experiment. The resistance of the iodide-fed pigs to disease, if one may interpret their high percentage survival⁴ in this way, is but slightly different from the resistance shown by 10 of the 17 control litters.

An experiment on rats, similar in its objects to that of Weiser and Zaitschek (14), has been reported by Maurer and Diez (9), of Munich. Two groups of four pregnant rats were fed during the gestation and lactation periods upon a diet of maize and milk. In the ration of one group the iodine content of the maize had been increased by a potassium iodide fertilizer, and that of the milk by supplementing with potassium iodide the ration of the dairy cows from which it was obtained. In this way, the iodine consumed by the test animals was raised to approximately twice that of the controls; that is, 4γ⁵ daily as compared with 2γ. Throughout the lactation period the body weights of the mother rats varied but little, but the average weight of the young of the mothers on the iodized ration exceeded

⁴ As another instance of the partiality shown the iodide-fed group in the experiment there were 11 pigs added to the control litters at or shortly after farrowing, and 22 added to the iodide-fed litters. No mention of this is made in the text, and no allowance made in the computation of mortality or survival percentages. An admission that the pens in which the control litters were confined received less sunshine than those of the iodide-fed litters, at a time when an antirachitic factor in the food or environment is an urgent necessity, and the value of warm sanitary quarters is at a premium, needs no comment.

⁵ γ=0.001 milligram.

that of the young of the mothers on the normal ration, so that at the end of three weeks the average difference in weight amounted to about 20 per cent. The average weights per litter at this time were 24, 26.5, 20, and 22 gm. for the test rats, and 18.5, 21, 16.5, and 18.5 gm. for the control rats. For two other female rats, potassium iodide was added to the normal ration at the rate of 20% daily. Neither the mothers nor their young were adversely affected by this much larger dosage of iodide, nor was the growth of the young appreciably different from that of the mothers on the untreated maize and milk. At 21 days of age the average weights of the young in these two litters were 19.5 and 21 gm. The evidence obtained in this experiment indicates a favorable effect of iodide feeding on growth at the lower level of intake, but statistically it is not competent to establish such an effect. Even less convincing are the chemical analyses of three young rats from each of four litters, two control and two test. These analyses are taken to prove profound changes in the moisture and ash contents of the rats as the result of iodide feeding.

Hanzlik, Talbot, and Gibson (6) have reported the results of feeding small doses of sodium iodide (about 1 mgm. daily) to young, growing rats in four experiments. The group-feeding method was employed, using three to five rats per group. The average data only are reported. The experimenters were concerned mainly with the question whether continued administration of iodide to animals will produce detrimental effects, having in mind the therapeutic treatment of thyroid troubles. Their results did not reveal any such effects, and from a consideration of the growth curves they concluded that—

the continued administration of iodide in small daily doses in food over long periods (covering from about one-seventh to seventh-twelfths of the span of life) to rats caused moderate though variable increases in weight and growth of the body in the majority of animals on complete dietary.

Even this conservative statement may be too positive, since in the third of the four experiments the control group exceeded the iodide-fed group in rate of growth throughout the period of observation, while in the first experiment the controls ultimately exceeded the test rats in median weight.

In apparent contrast to the experiments just reviewed, which have been interpreted to mean that a supplemental feeding of iodides to animals, either directly in their food or through the milk of the dam, may exert a favorable effect upon growth and well-being, may be cited a number of other experiments in which such indications are absent. Orr and Leitch (10) in their review of the literature on iodine in nutrition, describe briefly a number of experiments on swine performed at the Rowett Institute at Aberdeen, Scotland, in which the effect of supplemental iodine upon growth and reproduction was investigated. The results are interpreted as entirely negative, except in so far as they showed that no toxic effects on young pigs or on brood sows are likely to follow the administration of iodine up to that equivalent to 1 gm. of potassium iodide per head per day. Essentially negative results were also secured with calves.

Rothwell (11) investigated the value of potassium iodide for growing swine in an experiment at the Central Experimental Farm, Canada. A lot of five pigs received daily from 50 to 100 mgm. of potassium

iodide per head. Starting at an average weight of 55 pounds, their average rate of growth to a weight of 274 pounds was somewhat less than that of the control lot.

The Purdue Agricultural Experiment Station has reported⁶ the results of two years' work on the feeding of dried kelp to growing swine, with particular reference to its iodine content. Turrentine (13) reported the average composition of 29 samples of dried kelp from the Pacific coast, analyzed by the United States Department of Agriculture. Almost 40 per cent of this material consisted of inorganic salts, about one-half of which was sodium chloride. Its iodine content averaged 0.26 per cent and among the 29 samples ranged from 0.17 to 0.41 per cent. Turrentine voices an opinion not uncommonly held that—

it is preferable that the body be permitted to secure its iodine from some natural, vegetable carrier of iodine, by natural digestive processes of selection and elimination through a prolonged digestive period rather than from some chemical, highly soluble and of high penetrative powers, against which the natural protective and defensive agencies of the body are not able to prevail.

That sea plants are efficient as a source of iodine is indicated by the work of Hunter and Simpson (8), who found the iodine content of the thyroids of sheep in the Orkney Islands, which lived largely on seaweed during the winter, was extraordinarily high (1.05 per cent on the dry basis).

The Purdue experiments were of the ordinary description and have been reported in the usual way by averages only, so that it is not possible to assess the significance of the average by any statistical method. All lots of pigs in both experiments contained 10 animals each. In the first experiment a ration of ground corn 90 and fish meal 10 promoted an average daily gain of 1.55 pounds throughout 90 days of feeding. Substitution of 1 per cent of fish meal by 1 per cent of dried kelp resulted in the same average gain, but a similar substitution of 2 per cent gave an average gain of 1.63 pounds. With tankage or soybeans as the nitrogenous concentrate, the average daily gain of the lot receiving either 1 or 2 per cent of dried kelp exceeded that of the check lot, the averages being 1.23 and 1.54 pounds for tankage, and 0.94 and 1.04 pounds for soybeans. In the second year's experiment, a ration of shelled corn and meat scraps gave an average daily gain of 1.54 pounds in a 90-day feeding trial. When dried kelp replaced one-fifth of the meat scraps, the average daily gain was 1.49 pounds, and when it was fed "free choice," the average daily gain was 1.54 pounds. If the first experiment is taken to indicate a favorable effect of iodide as a supplement to the tankage or soybean rations, the second experiment may be explained on the basis of a possible higher iodine content of the basal feeds, making an iodide supplement ineffective. It seems logical, however, to conclude that both experiments are essentially negative, the apparently positive results in the first experiment being a not greatly improbable outcome of the operation of chance only.

In an experiment reported by Cameron and associates (3, 4), the feeding of sodium iodide to rats had no obvious effect upon growth, though the colloid in the thyroid gland was increased. Similarly

⁶ These results have been issued only as mimeographed sheets dated Nov. 20, 1928, and Sept. 27, 1929.

Hayden, Wenner, and Rucker (7) have observed that the addition of iodine to the drinking water of young rats, subsisting upon a diet of natural foods, decreased greatly the size of the thyroid apparently without affecting the rate of growth.

In evaluating the results of the foregoing experiments, account should be taken of the fact that positive results, indicating a definite nutritive effect of a dietary factor, can not be readily disproved by negative evidence unless some error in the experiments yielding positive results can be detected, or unless it can be shown that some alternative explanation has not been disposed of. But it is equally true that an experiment can be considered to possess a definite positive significance only when it satisfies some adequate method of statistical analysis. The fact that a test group of animals shows a larger average measurement than the group carried as a control is not proof that the difference in experimental treatment between the groups is responsible, since such an outcome would result once in every two trials by the operation of chance alone. Without further statistical analysis such a result can not be considered a positive one. Unfortunately most of the above-described experiments were not planned in such a way or reported in sufficient detail so that an adequate statistical analysis can be made of them. Hence, two interpretations of them are at hand, neither of which is demonstrably more probable than the other: (1) The apparently positive results were obtained with basal rations very low in iodine or with experimental animals on the verge of iodine undernutrition, while the negative results were obtained under conditions of adequate iodine nutrition; or (2) the occurrence of apparently positive results is purely the result of chance, such as would be obtained if the iodine supplement were without effect upon growth.

METHOD

Since it appeared to the writers that the difficulty in evaluating the significance of experiments on the supplementing value of iodides for the growth of animals was inherent in the group method of feeding *ad libitum*, it was decided to attack the problem by an entirely different method in which the control of experimental conditions is much more complete. The paired-feeding method was therefore used. In this method, each test animal has its own control animal, selected for equality of weight, sex, breeding, and condition, and treated in every way the same, including the quantity of food consumed, except for the dietary supplement whose effect upon growth it is desired to determine. Although in equalizing the food intakes of paired animals, the intake of one or the other is restricted from time to time as its pair mate refuses some of its ration, there is no basis for the belief that the comparability of the results obtained is at all impaired, or that their significance is modified. A ration deficient in any one constituent in the support of growth, should exhibit that deficiency for all intakes of food capable of supporting growth. For the same reason, the deficiency of corn proteins in tryptophane or of a corn ration in calcium is not dependent upon the amount of food consumed, but is evident at all levels of food intake consistent with growth.

TABLE 2.—*Composition of basal ration of pigs*

Feed	Proportions in which the feeds were mixed for the pigs weighing		
	Less than 100 pounds	From 100 to 150 pounds	Over 150 pounds
	<i>Pounds</i>		
Corn.....	76	84	92
Tankage.....	12	8	4
Linseed meal.....	6	4	2
Alfalfa meal.....	6	4	2
Salt.....	.5	.5	.5

The control animal in each pair is thus a control in all respects in so far as it is possible to make it so, with reference to its food requirements, its ability to grow, the character of the basal ration, and the amount of it consumed. Another marked advantage that the method possessed over the group-feeding method is that it capitalizes to the fullest extent the ability of the experimenter to select his animals for control and test treatment in order to assure the closest equality in performance under like conditions. The interpretation of the results by statistical methods is not in the slightest degree impaired by the most careful selection of experimental animals. However, with the group-feeding method the statistical analysis of the results is commonly made on the assumption, implied or admitted, that random selection only has been used, and in so far as the experimenter interferes with the randomness of the gains (or other results) secured, by his praiseworthy desire to balance properly his experimental groups, the applicability of the ordinary statistical method becomes less exact.

Thirteen carefully selected pairs of Poland-China pigs were used in this experiment. Their initial weights ranged from 57 to 76 pounds, though within any pair, the difference in initial weight did not exceed 4 pounds. The basal ration contained ground corn, tankage, linseed meal, alfalfa meal, and salt in proportions that were varied in accordance with the body weights of the pigs, as shown in Table 2. The chemical composition of samples of the feeds used, taken continuously throughout the experiment, is recorded in Table 3. The test on each pair was continued until at least one pig attained a weight of 175 pounds. The initial and final weights recorded in Table 4 are each the average of three weights taken on consecutive days. Intermediate weights were taken weekly on each pig. The essential data of the experiment are given in Table 4.

The potassium iodide was administered to each test pig of the 13 pairs once daily in water solution. An amount of this solution equivalent to 1 grain of elemental iodine was poured over the morning feed at feeding time.

TABLE 3.—*Chemical composition of the feeds used*

Feed	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	N-free extract	Gross energy
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Small calcs. per gm.</i>
Corn, yellow.....	86.64	8.27	3.39	1.38	2.43	71.17	3,830
Tankage.....	93.76	55.17	11.27	23.36	.64	3.32	4,304
Linseed meal.....	89.95	34.44	5.05	8.36	8.91	36.16	4,229
Alfalfa meal.....	88.98	15.31	2.03	7.13	27.29	37.22	3,939

TABLE 4.—Weights, gains or losses, and feed consumption of 13 pairs of pigs when one of each pair was fed potassium iodide as a mineral supplement to an otherwise adequate basal ration

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Final weight..... pounds	177	184	171	177	177	180	176	164	183	175
Initial weight..... pounds	76	76	74	73	69	70	71	64	65	67
Total gain..... do.	101	108	97	104	108	110	105	100	118	108
Test period..... days	148	148	169	160	141	141	162	162	134	134
Average daily gain..... pounds	.68	.73	.57	.62	.77	.78	.65	.62	.88	.81

WEEKLY GAINS OR LOSSES

Week	-4	-3	-4	-1	-2	-3	-3	-1	1	-3
1.....	4	5	5	4	3	6	6	4	5	6
2.....	4	5	4	3	6	6	5	4	0	0
3.....	4	5	6	5	5	6	5	4	7	3
4.....	5	6	6	5	5	5	6	6	7	5
5.....	6	7	5	6	7	7	6	5	8	6
6.....	4	7	6	9	7	7	5	7	6	5
7.....	8	5	5	3	3	5	5	4	5	6
8.....	4	4	6	6	7	6	6	4	5	6
9.....	10	8	8	7	8	10	7	8	7	9
10.....	2	5	-2	-3	3	3	1	1	1	1
11.....	8	7	7	11	7	7	-4	-5	9	8
12.....	6	6	-10	-14	7	7	1	1	6	6
13.....	-4	-3	-1	3	3	5	4	1	6	3
14.....	2	1	0	1	5	5	0	3	5	8
15.....	4	6	3	7	12	9	5	2	9	9
16.....	3	8	4	2	7	6	6	7	9	6
17.....	7	2	6	6	-3	0	5	6	9	10
18.....	6	5	8	4	10	7	8	0	9	7
19.....	6	6	5	9	4	8	5	9	* 9	* 13
20.....	10	8	* 7	5	* 10	* 5	8	6		
21.....	* 10	* 13	10	10			10	9		
22.....			3	5			3	9		
23.....			11	3			* 10	* 2		
24.....			* 5	* 14						
Total feed eaten..... pounds	493	493	543	543	519	519	504	504	494	494
Average daily ration..... pounds	3.33	3.33	3.22	3.22	3.68	3.68	3.11	3.11	3.68	3.68
Feed per pound of gain..... pounds	4.88	4.56	5.00	5.22	4.81	4.72	4.80	5.04	4.18	4.57

Item	Pair 6		Pair 7		Pair 8		Pair 9	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Final weight..... pounds	176	175	165	175	179	169	187	175
Initial weight..... do.	62	63	59	61	74	74	69	67
Total gain..... do.	114	112	106	114	105	95	118	108
Test period..... days	127	127	155	155	141	141	134	134
Average daily gain..... pounds	.90	.88	.68	.74	.74	.67	.88	.81

WEEKLY GAINS OR LOSSES

Week:	0	-1	-2	1	-3	-5	-2	-4
1.....	6	5	4	4	6	6	6	7
2.....	4	6	5	4	6	5	5	5
3.....	5	4	7	5	7	6	8	5
4.....	7	8	6	5	4	4	5	6
5.....	7	6	6	7	6	6	5	7
6.....	4	5	6	6	7	4	8	4

* 5 days in the last period.

TABLE 4.—Weights, gains or losses, and feed consumption of 13 pairs of pigs when one of each pair was fed potassium iodide as a mineral supplement to an otherwise adequate basal ration—Continued

WEEKLY GAINS OR LOSSES—Continued

Item	Pair 6		Pair 7		Pair 8		Pair 9	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Week—Continued.								
8.....	6	7	5	7	5	5	4	6
9.....	9	7	8	6	10	8	11	7
10.....	5	4	5	5	2	4	3	4
11.....	8	4	8	9	9	7	8	9
12.....	6	9	3	5	7	4	8	3
13.....	4	4	-6	-6	1	6	2	3
14.....	4	8	-6	2	7	4	4	7
15.....	11	6	2	1	0	2	10	8
16.....	7	10	6	6	2	1	9	6
17.....	13	11	5	8	4	6	7	9
18.....	* 8	* 9	11	4	9	6	11	6
19.....			7	7	5	4	* 5	* 10
20.....			7	8	* 11	* 12		
21.....			10	8				
22.....			* 9	* 12				
23.....								
24.....								
Total feed eaten..... pounds	483	483	519	519	470	470	518	518
Average daily ration..... do	3.80	3.80	3.35	3.35	3.33	3.33	3.86	3.86
Feed per pound of gain..... do	4.24	4.31	4.89	4.55	4.48	4.95	4.39	4.79

Item	Pair 10		Pair 11		Pair 12		Pair 13	
	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig	Check pig	Iodine-fed pig
Final weight..... pounds	175	179	173	182	176	173	180	178
Initial weight..... do	65	66	61	65	59	61	57	57
Total gain..... do	110	113	112	117	117	112	123	121
Test period..... days	141	141	169	169	162	162	162	162
Average daily gain..... pound	.78	.80	.66	.69	.72	.69	.76	.75

WEEKLY GAINS OR LOSSES

Week:								
1.....	-4	-2	-1	-4	-4	-2	-2	-1
2.....	7	6	5	6	6	6	8	7
3.....	5	5	5	7	7	3	3	2
4.....	7	7	4	-1	6	6	4	4
5.....	4	7	3	5	6	6	3	5
6.....	7	6	6	4	5	7	1	1
7.....	5	5	4	6	6	3	4	6
8.....	5	4	6	7	6	6	* 8	* 8
9.....	8	6	10	8	8	9	8	8
10.....	2	6	-1	0	2	0	0	2
11.....	8	8	8	10	9	8	3	4
12.....	6	4	-3	-3	1	4	4	2
13.....	5	6	-1	2	0	-2	6	4
14.....	4	5	-2	1	0	-1	5	5
15.....	10	10	4	3	7	7	5	4
16.....	8	8	4	3	4	5	5	7
17.....	11	11	10	8	7	6	9	7
18.....	-4	-5	5	4	5	4	8	8
19.....	5	8	5	7	6	11	8	8
20.....	* 11	* 8	9	9	9	6	13	9
21.....			9	6	9	8	4	6
22.....			7	7	7	6	6	7
23.....			10	7	* 5	* 6	* 10	* 10
24.....			* 6	* 16				
Total feed eaten..... pounds	520	520	561	561	571	571	529	529
Average daily ration..... do	3.69	3.69	3.32	3.32	3.52	3.52	3.26	3.26
Feed per pound of gain..... do	4.72	4.60	5.01	4.79	4.88	5.10	4.30	4.37

* 8 days in the last period.

Reference to Table 4 shows that in 7 of the 13 pairs the control pig made the more rapid gain, while in 6 pairs the iodide-fed pig gained more rapidly. A study of the gains from week to week confirms the negative character of the experiment. Of the 277 weekly comparisons between the gains of pair mates, 143.5 favored the check pig and 133.5 favored the pig receiving the iodide supplement. The ideal result, if chance alone operated, would be 138.5 comparisons favoring either the check or the test ration. The deviation of 5 from this ideal is considerably less than the standard deviation ($\sqrt{0.5 \times 0.5 \times 277} = 8.3$), and hence is insignificant as an indication of the operation of any but chance factors, not under control.

Under the conditions of this experiment, therefore, it seems clear that potassium iodide did not increase the growth-promoting value of the basal ration.

Thus the majority of experiments concerned with the effect of iodine supplements to natural rations on the growth of young animals not obviously afflicted with hypothyroidism indicate no beneficial effect, and those experiments that have been interpreted in a positive way are either statistically inadequate or have been demonstrably misinterpreted. It may be asked whether there is any good reason to believe that, in the absence of evidences of thyroid malfunction, iodine deficiency may nevertheless exist and hence iodine administration may favorably affect growth. The following facts are pertinent to this question, though they do not afford a complete answer to it:

(1) No other function of iodine in the metabolism of the higher animals has been demonstrated than its functional relationship to the thyroid gland.

(2) Thyroid enlargement seems to be an invariable accompaniment of a dietary deficiency of iodine. The absence of a hyperplastic thyroid may thus be fairly taken as an indication of a sufficient iodine supply.

(3) In normal animals, exhibiting no evidences of thyroid enlargement and no symptoms of hypothyroidism, the administration of thyroid material by mouth has in most experimental work been associated with a retardation rather than an acceleration of growth. This is in agreement with the known stimulating effect of thyroxine upon total and nitrogenous catabolism.

(4) In hypothyroidism, the administration of iodine simulates that of thyroid material, particularly with reference to its effect on the basal metabolic rate. The effect of the administration of both thyroid material and of iodine in normal animals is less regular than in hypothyroid animals, being subject apparently to individual constitutional or functional differences. The most characteristic effect of thyroid material, that is, its stimulating effect on heat production, is generally evident with normal adult animals, though with normal children it is frequently without effect (Topper and Cohen (12)). Iodide administration to normal animals has been observed to increase basal metabolism, to be without effect, and to decrease it, though in all cases the changes were slight.

There is thus no compelling reason to expect that small addenda of iodide to the ration will affect animal growth in the absence of thyroid hypofunctioning, nor to assume, as Evvard and Culbertson (5, p. 193) do, that

inasmuch as goitre represents fairly advanced stages of the disease it is easy to see that there may be a shortage of iodine in the ration sufficient to prevent adequate nutrition and yet not so great a deficiency as to develop goitrous pathology.

SUMMARY

A critical analysis of published experiments on the effect of supplemental iodides on the growth of animals not obviously suffering from

hypothyroidism, shows that the majority indicate no beneficial effect and that those experiments which have been interpreted in a positive way are either statistically inadequate or have been demonstrably misinterpreted.

In a paired-feeding experiment involving 13 pairs of Poland-China pigs, weighing initially from 57 to 76 pounds and finally about 175 pounds, no evidence was obtained that the administered potassium iodide, equivalent to 1 grain of iodine daily, in any way affected the rate of growth. In 7 of the 13 pairs the control pig made the more rapid gain. The basal ration used contained ground yellow corn, tankage, linseed meal, alfalfa meal, and sodium chloride.

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INFLUENCE OF SMALL QUANTITIES OF POTASSIUM IODIDE ON THE ASSIMILATION OF NITROGEN, PHOSPHORUS, AND CALCIUM IN THE GROWING PIG¹

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PREVIOUS INVESTIGATION

In 1925, Kelly (6),² of the Rowett Research Institute at Aberdeen, Scotland, published the results of an experiment upon four growing pigs to determine the effect of potassium iodide upon the metabolism of nitrogen, calcium, and phosphorus. The results obtained were interpreted as indicating definitely that potassium iodide in the amount used increased the retention of both nitrogen and phosphorus, and less definitely of calcium. The experiment was carefully planned and executed, and the results were, for three of the four pigs, adequately controlled. In fact, for three of the pigs two methods of control were adopted, one in which each pig was a control upon itself in a preperiod and a post period of no iodide supplements, the other in which a control pig, receiving no iodide supplement at any time, was carried along on the same amount of the basal ration that the test pig received. The fourth pig was the control pig for one of the other three and was later given three increasing doses of potassium iodide in as many periods. In this case the control was hardly adequate.

With only three or four experimental animals it may be urged that the data should consistently support one interpretation before a definite conclusion is established. But in the experiment of Kelly the data are not consistent throughout. In the first experiment, the experimental pig showed greater retention of nitrogen and calcium in the potassium-iodide period than in the control period and a smaller retention of phosphorus. A markedly greater retention of all three elements occurred in the postperiod than in any of the preceding periods. However, Kelly does not use the results of this period in his interpretation because "during this period the animal had a tendency to eat its feces, thus rendering the results unreliable." The discarding of one-third of the data on this pig should be based upon something more than a casual observation of this description, particularly when there is no obvious reason why coprophagy should have been confined to one experimental animal in one experimental period.

In the second experiment, the experimental pig stored more nitrogen, calcium, and phosphorus in the potassium-iodide period than in either the preceding or the following control periods. In the third experiment, the test pig retained more of all three elements in the potassium-iodide period than in the preperiod, but less of phosphorus and of calcium than in the postperiod. The fourth experimental pig showed no distinct increase in the assimilation of nitrogen, calcium, and phosphorus in periods in which 3 or 6 mgm. of potassium iodide

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² Reference is made by number (italic) to "Literature cited," p. 87.

were given daily, but when the dose was raised in a succeeding period to 0.5 gm. daily, an enormous increase in the storage of phosphorus, a moderate increase in the storage of nitrogen, and a slight and probably insignificant increase in the storage of calcium occurred. No postperiod was run in this case.

The interpretation that Kelly gives to his data was based less upon such relations as those just described than upon a comparison of test and control pigs, attention being almost entirely confined to the preperiods and the postassium-iodide periods. Since the data are few and are not consistent throughout, the conclusions deduced are not convincing. In explaining his conclusion that iodine administration increases nitrogen retention Kelly says, "the increased retention of nitrogen after the addition of iodine, observed in all these experiments, is an index of the inducement of better growth probably due to the stimulation of the thyroid gland by the iodine." But, as Kelly himself points out, the observed increases in nitrogen retention associated with iodide administration, were, in agreement with a number of other experiments, accompanied in general by increased urinary excretion of nitrogen and hence were generally the result of a decreased fecal excretion of nitrogen. Hence, if there is a causal relation between the observed increases in nitrogen retention and the iodine administered, one must assume that potassium iodide stimulates the digestive enzymes, or in other ways induces a more complete digestion. But a number of investigations, including those of Loevenhart and Peirce (7) on liver and pancreatic lipase, those of Berczeller and Freud (1, 2) on malt and salivary diastase, and particularly those of Clifford (3, 4, 5) on ptyalin and pepsin, have shown with remarkable unanimity that iodides, and particularly potassium iodide, retard the action of digestive enzymes, or totally inactivate them, in all concentrations producing any effect at all. Also, Magee and Reid (8) have observed that the addition of small quantities of potassium iodide to the nutrient solution containing excised rabbit intestine increases the rate and amplitude of the intestinal contraction, and that the motility of the intestines from rabbits receiving iodide was greater than that of the controls. In the intact animal it is known that large doses of iodides cause diarrhea. These experimental findings are not consistent with the view that potassium iodide exerts a favorable effect upon digestion, and hence they suggest that the observed relation in Kelly's experiments between iodide dosage and decreased fecal excretion of nitrogen is not a causal one.

EXPERIMENTAL METHODS

The uncertainties that appear to exist in the interpretation of Kelly's data, and the objections that may be raised against the acceptance of his conclusions, render a repetition of the work desirable. Accordingly, the writers undertook metabolism studies on five pigs. A larger number of pigs could not be handled simultaneously with the equipment available. Hence, in order to make the most of the experiment, each pig was allowed to serve as a control upon itself in a preperiod and a postperiod in which no iodide was administered. In the test period each pig received 0.248 gm. of potassium iodide. This is the dosage used in Kelly's experiments. Each of the three periods lasted 14 days, but the collections of urine and feces were composited for analysis every 7 days. The first collection period was preceded by a

3-day period in the metabolism crates on the same amounts of food consumed during the subsequent seven days.

The pigs were crossbreds between the Poland-China and the Duroc-Jersey breeds. Their initial weights were 120, 108, 113, 111, and 108.5 pounds, respectively. Three of them were gilts and two were barrows.

The basal ration was the same in composition throughout all periods, and consisted of 79.5 parts of ground yellow corn, 15 parts of soybean-oil meal, 4 parts of linseed meal, 1 part of cod-liver oil, and 0.5 part of NaCl per 100 parts of ration. In addition, each pig received daily 5 gms. of calcium in the form of $\text{Ca}_3(\text{PO}_4)_2$. The intake of food was somewhat smaller in the fore period than in the test and after periods, but in the latter two periods it was nearly the same.

TABLE 1. - *Body weights, and food consumption of the pigs*

PIG 32-30 S ^a					
Period	Length of period	Initial weight	Final weight	Gain	Feed consumed daily
	Days	Pounds	Pounds	Pounds	Grams
Fore 1	7	120.0	131.0	11.0	1,800
Fore 2	7	131.0	138.5	7.5	1,980
Test 1	7	138.5	151.0	12.5	2,134
Test 2	7	151.0	161.5	10.5	2,160
After 1	7	161.5	175.5	14.0	2,180
After 2	7	175.5	184.5	9.0	2,160
PIG 24-3 S					
Fore 1	7	108.0	118.5	10.5	1,700
Fore 2	6 ^b	118.5	122.5	4.0	1,558
Test 1	5 ^c	122.5	131.5	9.0	1,836
Test 2	7	131.5	137.5	6.0	2,040
After 1	7	137.5	155.0	17.5	2,040
After 2	7	155.0	160.5	5.5	2,040
PIG 6-6 S					
Fore 1	7	113.0	127.5	14.5	1,800
Fore 2	7	127.5	135.5	8.0	1,980
Test 1	7	135.5	148.0	12.5	2,134
Test 2	7	148.0	161.5	13.5	2,160
After 1	7	161.5	172.5	11.0	2,160
After 2	7	172.5	177.5	5.0	2,160
PIG 6-9 B					
Fore 1	7	111.0	121.5	10.5	1,600
Fore 2	7	121.5	130.0	8.5	1,760
Test 1	7	130.0	138.5	8.5	1,847
Test 2	7	138.5	150.5	12.0	1,920
After 1	7	150.5	162.0	11.5	2,000
After 2	7	162.0	172.5	10.5	2,000
PIG 39-30 B					
Fore 1	7	108.5	121.0	12.5	1,800
Fore 2	7	121.0	128.5	7.5	1,980
Test 1	7	128.5	140.5	12.0	2,134
Test 2	7	140.5	152.0	11.5	2,160
After 1	7	152.0	163.5	11.5	2,160
After 2	7	163.5	175.5	12.0	2,160

^a S=sow and B=barrow.

^b This pig vomited part of its food on the fourth day of the period. The urine and feces were discarded for this day. For the following two days the food intake was irregular.

^c The pig vomited on the first and fifth days of this period, and on these days the collections of urine and feces were discarded. The food intake on the second day was only 1,020 gms.

EXPERIMENTAL RESULTS

The food intakes of the pigs and the weight changes are summarized in Table 1. There is no indication that the iodide supplement during the test period affected the rate of growth of these young pigs. The average gains for the five pigs in the successive 14-day periods were 18.9, 21.6, and 21.5 pounds, respectively.

TABLE 2.—*The fecal excretion and the digestibility of nitrogen*

PIG 32-30

Period	Average daily fresh feces voided	Average daily feces on dry basis	Dry sub- stance in fresh feces	Digesti- bility of nitrogen
	Grams	Grams	Per cent	Per cent
Fore 1.....	526	189	36.0	80.2
Fore 2.....	622	228	36.7	76.4
Test 1.....	788	318	40.3	71.1
Test 2.....	839	286	34.1	75.3
After 1.....	623	265	42.4	80.0
After 2.....	567	215	37.8	83.5

PIG 24-3

Fore 1.....	595	204	34.2	76.9
Fore 2.....	622	241	38.8	70.4
Test 1.....	498	217	43.5	75.5
Test 2.....	660	250	37.9	77.0
After 1.....	545	222	40.7	81.2
After 2.....	511	197	38.6	82.9

PIG 6-6

Fore 1.....	628	212	33.7	80.5
Fore 2.....	771	258	33.4	78.4
Test 1.....	787	231	29.4	82.8
Test 2.....	701	258	36.8	82.0
After 1.....	580	165	28.4	88.8

PIG 6-9

Fore 1.....	551	196	35.5	78.4
Fore 2.....	728	219	30.0	77.4
Test 1.....	705	251	35.6	78.9
Test 2.....	615	219	35.6	82.6
After 1.....	542	221	40.8	82.0
After 2.....	503	164	32.8	85.6

PIG 39-30

Fore 1.....	590	211	35.8	77.7
Fore 2.....	770	259	33.6	74.3
Test 1.....	772	278	36.0	73.4
Test 2.....	668	243	36.4	78.7
After 1.....	535	216	40.4	79.0
After 2.....	575	197	34.3	82.1

In Table 2 the data concerning the fecal excretion and the digestibility of nitrogen are given. In successive 7-day periods the average daily weights of dry feces were 202, 241, 259, 251, 218, and 193 gm.; the average percentages of dry matter in the feces were 35.0, 34.5, 37.0, 36.2, 38.5, and 35.8; and the average coefficients of digestibility of nitrogen were 78.7, 75.4, 76.3, 79.3, 82.2, and 83.5. In each series of values the last figure is the average of four instead of five pigs,

since the last collection of feces for pig 6-6 was not analyzed, the corresponding urine collection having been thrown away by mistake. The data do not indicate any clearly evident effects of the iodide feeding, though they suggest that the weight of dry feces may have been somewhat increased and the percentage of moisture somewhat decreased in the test periods.

TABLE 3.—*Nitrogen balances of the pigs*

[All weights in grams]

PIG 32-30

Period	Intake	Excreted in—		Total output	Balance
		Feces	Urine		
Fore.....	38.97	7.71	17.39	25.10	+13.87
	42.87	10.10	22.01	32.11	+10.76
Test.....	46.21	13.36	23.00	36.36	+9.85
	46.76	11.53	23.18	34.71	+12.05
After.....	46.76	9.33	22.38	31.71	+15.05
	46.64	7.72	21.65	29.37	+17.27

PIG 24-3

Fore.....	36.80	8.49	18.04	26.53	+10.27
	43.74	9.99	15.71	25.70	+8.04
Test.....	39.75	9.75	20.13	29.88	+9.87
	44.17	9.78	23.30	33.08	+11.09
After.....	44.17	8.29	22.75	31.04	+13.13
	44.05	7.54	24.90	32.53	+11.52

PIG 6-6

Fore.....	38.97	7.60	11.34	18.94	+20.03
	42.97	9.25	22.68	31.93	+11.04
Test.....	46.21	7.96	21.26	29.22	+16.99
	46.76	8.41	21.43	29.84	+16.92
After.....	46.76	5.26	13.08	18.34	+28.42

PIG 6-9

Fore.....	34.64	7.49	16.80	24.29	+10.35
	38.10	8.62	17.51	26.13	+11.97
Test.....	41.08	8.65	20.28	28.93	+12.15
	41.57	7.23	21.15	28.38	+13.19
After.....	43.30	7.80	20.31	28.11	+15.19
	41.76	6.02	19.53	25.55	+16.21

PIG 39-30

Fore.....	38.97	8.68	19.30	27.98	+10.99
	42.87	11.01	19.90	30.91	+11.96
Test.....	46.20	12.31	23.38	35.69	+10.51
	46.76	10.00	22.08	32.08	+14.68
After.....	46.76	9.81	23.41	33.22	+13.54
	46.64	8.36	19.88	28.24	+18.40

TABLE 4.—Calcium balances of the pigs

[All weights in grams]

PIG 32-30

Period	Intake	Excreted in—		Total output	Balance
		Feces	Urine		
Fore.....	5.63	2.28	0.06	2.34	+3.29
	5.76	2.76	.07	2.83	+2.93
Test.....	5.87	3.80	.07	3.87	+2.00
	5.89	3.97	.03	4.00	+1.89
After.....	5.89	4.10	.04	4.14	+1.75
	5.87	3.44	.06	3.50	+2.37

PIG 24-3

Fore.....	5.56	2.69	0.13	2.82	+2.74
	5.45	3.18	.11	3.29	+2.16
Test.....	5.65	3.45	.10	3.55	+2.10
	5.80	4.55	.08	4.63	+1.17
After.....	5.80	3.71	.07	3.78	+2.02
	5.78	3.39	.05	3.44	+2.34

PIG 6-6

Fore.....	5.63	2.72	0.07	2.79	+2.84
	5.76	3.16	.07	3.23	+2.53
Test.....	5.87	2.92	.08	3.00	+2.87
	5.89	3.52	.06	3.58	+2.31
After.....	5.89	2.27	.04	2.31	+3.58

PIG 6-9

Fore.....	5.48	2.26	0.06	2.32	+3.16
	5.60	2.65	.06	2.71	+2.89
Test.....	5.70	3.22	.05	3.27	+2.43
	5.71	3.61	.03	3.64	+2.07
After.....	5.77	3.94	.06	4.00	+1.77
	5.75	2.92	.05	2.97	+2.78

PIG 39-80

Fore.....	5.63	2.58	0.11	2.69	+2.94
	5.76	3.47	.15	3.62	+2.14
Test.....	5.87	3.92	.06	3.98	+1.89
	5.89	3.98	.05	4.03	+1.86
After.....	5.89	3.59	.07	3.66	+2.23
	5.87	3.45	.05	3.50	+2.37

TABLE 5.—*Phosphorus balances of the pigs*

[All weights in grams]

PIG 32-30

Period	Intake	Excreted in—		Total output	Balance
		Feces	Urine		
Fore.....	7.91	3.63	1.16	4.79	+3.12
	8.46	4.27	1.49	5.76	+2.70
Test.....	8.93	5.95	1.31	7.26	+1.67
	9.01	5.65	1.27	6.92	+2.09
After.....	9.01	6.84	1.58	8.42	+1.59
	9.12	5.91	1.52	7.43	+1.69

PIG 24-3

Fore.....	7.60	3.35	1.33	4.68	+2.92
	7.50	3.92	1.31	5.23	+2.33
Test.....	8.02	4.41	1.46	5.87	+2.15
	8.65	5.80	1.25	7.05	+1.60
After.....	8.65	5.19	1.40	6.59	+2.06
	8.75	4.96	1.86	6.82	+1.93

PIG 6-6

Fore.....	7.91	4.06	0.94	5.00	+2.91
	8.46	4.81	1.11	5.92	+2.54
Test.....	8.93	4.74	1.52	6.26	+2.67
	9.01	6.11	.76	6.87	+2.14
After.....	9.01	3.83	1.09	4.92	+4.09

PIG 6-9

Fore.....	7.29	3.40	1.12	4.52	+2.77
	7.78	3.98	1.15	5.13	+2.65
Test.....	8.21	4.91	1.44	6.35	+1.86
	8.28	5.00	1.13	6.13	+2.15
After.....	8.52	5.90	1.28	7.18	+1.34
	8.62	4.57	1.30	5.87	+2.75

PIG 39-30

Fore.....	7.91	3.99	0.90	4.89	+3.02
	8.46	4.94	1.57	6.51	+1.95
Test.....	8.93	5.34	1.40	6.74	+2.19
	9.01	5.88	1.55	7.23	+1.78
After.....	9.01	6.51	1.30	6.81	+2.20
	9.12	5.91	1.41	7.32	+1.80

TABLE 6.—*Summary of the average daily retention of nitrogen, calcium, and phosphorus by individual pigs*

NITROGEN RETENTION IN GRAMS PER DAY

Period	Pig 32-30	Pig 24-3	Pig 6-6	Pig 6-9	Pig 39-30	Average
Fore.....	13.87	10.27	20.03	10.35	10.99	13.10
	10.76	8.04	11.04	11.97	11.96	10.75
Test.....	9.85	9.87	16.99	12.15	10.51	11.87
	12.05	11.09	16.92	13.19	14.68	13.59
After.....	15.05	13.13	28.42	15.19	13.54	17.07
	17.27	11.52		16.21	18.40	15.85

CALCIUM RETENTION IN GRAMS PER DAY

Fore.....	3.29	2.74	2.84	3.16	2.94	3.00
	2.93	2.16	2.53	2.89	2.14	2.53
Test.....	2.00	2.10	2.87	2.43	1.89	2.26
	1.89	1.17	2.31	2.07	1.86	1.86
After.....	1.75	2.02	3.68	1.77	2.23	2.27
	2.37	2.34		2.78	2.37	2.46

PHOSPHORUS RETENTION IN GRAMS PER DAY

Fore.....	3.12	2.92	2.91	2.77	3.02	2.95
	2.70	2.33	2.54	2.65	1.95	2.43
Test.....	1.67	2.15	2.67	1.89	2.19	2.11
	2.09	1.60	2.14	2.15	1.78	1.95
After.....	.59	2.06	1.00	1.34	2.20	2.06
	1.69	1.93		2.75	1.80	2.04

The complete balance data for nitrogen, calcium, and phosphorus are summarized in Tables 3, 4, and 5. The balances (retentions) only have been summarized in Table 6. There are no consistent differences between the data of the test period and those of the fore and after periods with respect to either nitrogen or phosphorus, and hence no suspicion that the administration of potassium iodide has affected in any way the utilization of these elements. With respect to calcium there is a suggestion of an adverse effect of potassium iodide, particularly if it is permissible to assume that such an effect may extend into the first week of the after period. The data for pigs 39-30 and 24-3 offer the clearest support for this suggestion; those for pigs 32-30 and 6-9 may also be given the same significance, though with less certainty, while the data for pig 6-6 are not clearly amenable to the same interpretation.

• The experiment affords no support for Kelly's conclusion that potassium iodide administration favorably influences the metabolism of the pig.

SUMMARY

In metabolism experiments upon five pigs, in which potassium iodide was administered for 14 days in doses of 0.248 gm., and in which 14-day control periods preceded and followed this régime, there was no evidence that the utilization of either nitrogen, calcium, or phosphorus was favorably affected. There was some indication that the retention of calcium was adversely affected. No effect of the iodide administration on the rate of growth of the pigs was evident.

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VARIATIONS IN CONTENT OF SUGARS AND RELATED SUBSTANCES IN OLIVES¹

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INTRODUCTION

In connection with a study of the composition of California olives it was found necessary to know something of the nature and amounts of the sugars and to select suitable methods for their determination. The fruit of olives of 17 varieties grown in California was therefore examined for the purpose of ascertaining (1) the amount and nature of the reducing substances in the flesh, and (2) the relation of these substances to the maturity and variety of the fruit.

REVIEW OF THE LITERATURE

Few references to the amount or nature of the sugars in olives appear in the literature. Power and Tutin,² studying the alcoholic extract of dried olive bark and leaves, reported the presence of sugar yielding *d*-phenylglucosazone. Bourquelot and Vintilescu³ reported the presence in olive flesh of a phenolic compound causing Fehling's solution to indicate more than the actual amount of sugar. Scurti and Tommasi⁴ in an attempt to explain the formation of oil in olives found no notable quantities of carbohydrates.

MATERIAL STUDIED

The varieties studied may be divided into three groups: (1) Varieties well established in California; Mission, Manzanillo, Sevillano, and Ascolano. These varieties are grown primarily for pickling and only the Mission is used for oil making. (2) Several varieties less widely grown—the Barouni, Columbella, Lucca, Nevadillo, Picholine, and Uvaria. (3) Varieties but recently introduced⁵ into California—the Bidh el Hammam, Chemlali, Chitoni, P. I. G. 27172, Saiali Magloub, Salome, and S. P. I. 27173.

The relation between composition and maturity was studied in the Ascolano, Columbella, Manzanillo, Mission, Nevadillo, and Uvaria. In each of these varieties a series of samples was obtained from a single tree except in two instances where fruit was no longer available on the selected tree, and final samples were taken from neighboring

¹ Received for publication July 24, 1929; issued June, 1930.

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⁴ SCURTI, F., and TOMMASI, G. SULLA FORMAZIONE DEL GRASSO NEI FRUTTI OLEAGINOSI. [FORMATION OF FAT IN OLEAGINOUS FRUITS (OLIVES)]. *Ann. R. Istit. Chim. Agr. Sper. Roma* (11) 4: 253-286. 1910. [Abstract in *Jour. Soc. Chem. Indus.* 30: 1021. 1911. (Original not seen.)]

⁵ The Chico, Calif., Plant Introduction Garden of the Bureau of Plant Industry, United States Department of Agriculture kindly cooperated by furnishing these varieties.

trees. In all cases an effort was made to minimize sampling errors by picking approximately half of each sample from opposite sides of the tree, and by picking all the fruit on the branches or twigs chosen at each sampling. The picking dates, at somewhat irregular intervals, ranged from October 1, 1927, to February 13, 1928, according to the maturing habits of the variety and the lateness at which sufficient fruit remained on the trees. In most instances the first picking was made when the fruit had reached about three-fourths its full size, was distinctly immature for pickling and of low oil content, and the last picking when it was rapidly falling from the trees, past its prime for oil making.

METHODS OF ANALYSIS

AMOUNTS OF SUGAR

The sugars were extracted from ground samples of the flesh by boiling with distilled water in a 500 c. c. volumetric flask for approximately 30 minutes, making up nearly to the mark, allowing to stand overnight, and then filling up to the mark. The decoction was then thoroughly shaken and strained through dry cheesecloth, and portions of the filtrate were set aside for clarification. Determinations of the amounts of sugars present were made by the picric-acid reduction method of Thomas and Dutcher.⁶ Reducing sugars present both before and after hydrolysis were reported as dextrose in all cases, and regarded as simple and total sugars, respectively.

NATURE OF THE REDUCING SUBSTANCES

On a few samples dry basic lead acetate, subsequently removed by sodium oxalate, was compared as a clarifying agent with mercuric nitrate, removed by sodium bicarbonate and powdered zinc. The nature of the reducing substances present was further studied in a few samples by adding one-fifth of a commercial compressed yeast cake to a 50 c. c. portion of the filtrate prepared as previously described and brought to 37° C. The sample was thoroughly stirred to break up the yeast cake and then incubated for 20 minutes at 37° F., being shaken at 5-minute intervals. It was then clarified and examined in the usual way.

EXPERIMENTAL DATA

CLARIFICATION

Basic lead acetate was found to leave a distinctly greater reducing power in the water extract than did mercuric nitrate, as shown in Table 1. Not only were the percentages of reducing substances, both before and after hydrolysis, greater when lead was used, but the increase on hydrolysis by picric acid was also always greater. This would indicate either that the mercury removed some sucrose or that the lead failed to remove nonsugar substances, of which hydrolysis by picric acid produced or increased the reducing power. From the

⁶ THOMAS, W., and DUTCHER, R. A. THE COLORIMETRIC DETERMINATION OF CARBOHYDRATES IN PLANTS BY THE PICRIC ACID REDUCTION METHOD. I. THE ESTIMATION OF REDUCING SUGARS AND SUCROSE. *Jour. Amer. Chem. Soc.* 46: 1662-1669. 1924.

results obtained from yeast treatment it is assumed that the lead failed to remove completely all the nonsugar substances that had reducing power either before or after hydrolysis.

TABLE 1.--Comparison of basic lead acetate and mercuric nitrate as clarifying agents in the determination of sugars in olives

[Reducing substances and sucrose reported as dextrose]

Variety	Date picked	Clarifying agent	Reducing substances before hydrolysis	Reducing substances after hydrolysis	Sucrose
	1927		Per cent	Per cent	Per cent
Ascolano.....	Oct. 4	Lead.....	5.00	6.92	1.92
		Mercury.....	4.62	5.42	.80
		Difference.....	38	1.50
Columbella.....	Oct. 1	Lead.....	3.46	4.34	.88
		Mercury.....	1.58	1.70	.12
		Difference.....	1.88	2.64
Manzanillo.....	do.....	Lead.....	4.52	5.18	.66
		Mercury.....	2.04	2.28	.24
		Difference.....	2.48	2.90
Mission No. 1.....	do.....	Lead.....	4.20	4.88	.68
		Mercury.....	1.88	2.16	.28
		Difference.....	2.32	2.72
Mission No. 3.....	Oct. 4	Lead.....	6.40	9.20	2.80
		Mercury.....	4.92	5.62	.70
		Difference.....	1.48	3.58
Do.....	Nov. 1	Lead.....	4.42	5.48	1.06
		Mercury.....	3.42	4.02	.60
		Difference.....	1.00	1.46
Nevadillo No. 1.....	Oct. 1	Lead.....	4.14	5.02	.88
		Mercury.....	1.62	2.02	.40
		Difference.....	2.52	3.00
Sevillano No. 2.....	Oct. 4	Lead.....	6.32	8.00	1.68
		Mercury.....	5.54	6.90	1.36
		Difference.....	.78	1.10
Uvaria.....	Oct. 1	Lead.....	2.98	6.02	3.04
		Mercury.....	1.04	1.20	.16
		Difference.....	1.94	4.72

YEAST TREATMENT

The yeast treatment was given to six olive samples, to a 0.4 per cent dextrose solution, and to distilled water, none of which had reducing power after clarification with mercuric nitrate. Three portions of one sample were clarified in three ways, namely, one with mercuric nitrate, one with basic lead acetate, and one with yeast followed by basic lead acetate. The results obtained are given in Table 2.

TABLE 2.—*Reducing substances remaining in an extract from olives after fermentation with yeast, and after clarification with different defecating agents*

[Reducing substances reported as dextrose]

Treatment	Reducing substances—	
	Before hydrolysis	After hydrolysis
	Per cent	Per cent
Clarified with mercuric nitrate.....	1.58	1.70
Clarified with basic lead acetate.....	3.46	4.34
Treated with yeast, then clarified with basic lead acetate.....	2.42	3.02

It is apparent from Tables 1 and 2 that clarification by basic lead acetate for the determination of sugars in olives by the picric-acid method appears unsatisfactory. On the other hand, reducing substances and substances having reducing power after hydrolysis that are left in the extract by mercuric nitrate appear to be limited to fermentable sugars. Mercuric nitrate was therefore used in the routine determinations of simple and total reducing substances. In the remainder of this paper the substances so determined will be considered as sugars.

RATIO OF SIMPLE TO TOTAL SUGARS

The ratios of simple to total sugars, expressed as dextrose, determined in several sets of samples representing the extremes of maturity studied are given in Table 3. The early-season mean ratio is 0.86, ranging from 0.80 to 0.89. The late-season mean ratio is 0.77, ranging from 0.67 to 0.94. Excluding the two samples not from the trees originally selected, the late-season mean ratio is 0.74, ranging from 0.71 to 0.77. The irregularity of the values obtained from samples not from the tree originally selected as shown in Table 3 and also in Table 4 indicate that the sampling error was probably large. From the ratios given it appears that the amounts of sugars principally or entirely sucrose, hydrolyzed by heating with picric acid, constitute usually from 15 to 25 per cent of the total reducing substances.

TABLE 3.—*Ratio of simple to total sugars in olives*

Variety	Date picked	Ratio
Ascolano.....	(Oct. 4, 1927.....	0.85
	Jan. 3, 1928.....	.77
Manzanillo.....	(Oct. 1, 1927.....	.89
	Feb. 13, 1928.....	.76
Mission No. 1.....	(Oct. 1, 1927.....	.87
	Feb. 13, 1928 *.....	.67
Mission No. 3.....	(Oct. 4, 1927.....	.88
	Nov. 1, 1927.....	.76
Nevadillo.....	Jan. 2, 1928.....	.71
	(Oct. 1, 1927.....	.80
	Feb. 13, 1928 *.....	.94

* Fruit not from tree originally selected.

MATURITY AND SUGAR CONTENT

Only the total sugars were considered in comparing different stages of maturity and different varieties. In Table 4 are shown the per-

centages of total sugars in six varieties of olives picked on the dates indicated. In addition to the figures as determined on the fresh basis there are given the corresponding figures after calculation to the dry basis.

TABLE 4.—*Total sugars^a in olives during ripening*

Variety	Date picked	Total sugars on—		Grams per 100 grams pits (fresh basis)
		Fresh basis	Dry basis	
		<i>Per cent</i>	<i>Per cent</i>	
Ascolano	Oct. 4, 1927	5.42	16.9	36.2
	Nov. 1, 1927 ^b	2.28	8.8	17.6
	Nov. 10, 1927	5.54	21.2	54.8
	Jan. 3, 1928	5.66	16.5	36.5
Columbella	Oct. 1, 1927	1.70	6.6	5.1
	Nov. 5, 1927	2.70	11.0	8.4
	Dec. 13, 1927	3.25	12.7	12.4
	Oct. 1, 1927	2.28	8.5	11.2
Manzanillo	Nov. 5, 1927	2.68	10.2	11.5
	Dec. 13, 1927	4.22	13.5	18.7
	Feb. 13, 1928	2.68	7.1	16.6
	Oct. 1, 1927	2.16	6.5	5.4
Mission No. 1	Nov. 5, 1927	2.70	8.6	10.0
	Dec. 13, 1927	3.82	10.6	16.4
	Feb. 13, 1928 ^c	3.56	6.7	6.3
	Oct. 4, 1927	5.62	15.4	15.2
Mission No. 3	Nov. 1, 1927	4.02	12.1	14.7
	Jan. 3, 1928	4.66	9.3	11.5
	Oct. 1, 1927	2.02	5.2	8.9
	Nov. 5, 1927	3.02	7.6	14.4
Nevadillo No. 1	Dec. 13, 1927	2.68	7.0	12.8
	Feb. 13, 1928 ^c	.96	1.9	4.6
	Oct. 1, 1927	1.20	5.0	1.6
	Nov. 5, 1927	2.25	10.2	3.6
Uvaia	Dec. 13, 1927	2.30	6.7	3.0

^a Reported as dextrose

^b Not considered in mean, Tables 5 and 6.

^c Not from tree originally selected.

The trend, especially in the figures on the dry basis, is for the sugar content to increase in October and November, and to decrease in December and January.

Previous investigations⁷ having shown that through the range of maturity studied the average pit weight remains constant, the figures were also calculated in terms of grams of sugars per 100 g. of pits in order to indicate the absolute amounts present. Since this eliminates the distortion of percentage resulting from coincident marked changes in the oil content of the flesh, the quantitative variations are different, but the direction of the trend is the same.

Variations apparently resulting from varietal differences in maturing time have been adjusted in Figure 1 by shifting some of the curves horizontally to bring the maximum values observed to a common vertical axis. The means of the values grouped according to vertical axes were used as a basis for the smoothed curves.

VARIETY AND SUGAR CONTENT

In view of the relationship which appeared to exist between maturity and sugar content, the varieties were compared with respect to

⁷ NICHOLS, P. F. RECENT INVESTIGATIONS ON OLIVE OIL. Ann. Tech. Conf. Calif. Olive Assoc. (San Francisco) Proc. 6: 73-84. 1927.

sugar content in early season, in late season, and for the entire season. In Table 5, the relative positions of the varieties are arranged according to mean sugar content on the fresh basis for the entire season. It will be noted, however, that the positions of varieties differ slightly

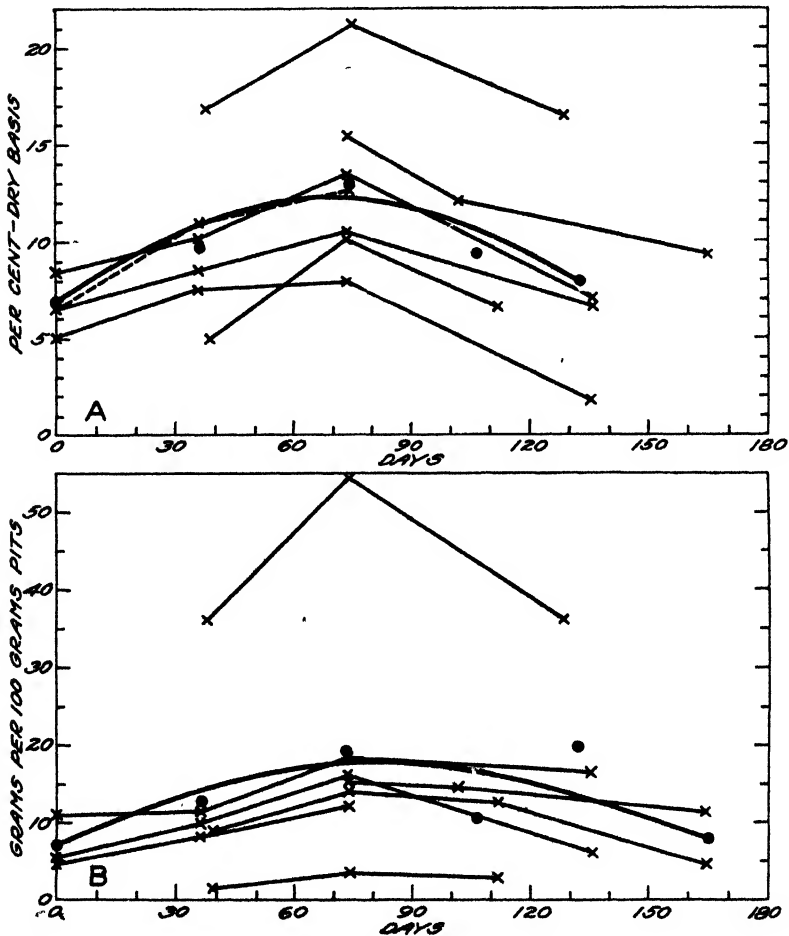


FIGURE 1.—A, Total sugars in olives during ripening, expressed as per cent on a dry basis. Maximum values adjusted to same vertical axis. Mean values indicated by circles and heavy curved line. B, Total sugars in olives during ripening, expressed as grams per hundred grams of pits. Maximum values adjusted to same vertical axis. Mean values indicated by circles and heavy curved line

from that order in the early and late seasons. The same is true in Table 6, in which the varieties are similarly arranged with respect to mean sugar content calculated on the dry basis. In almost all cases, however, the relative position remains approximately the same.

TABLE 5.—*Total sugars in olives of different varieties expressed in percentage on a fresh basis, in the early, late, and entire season*

Variety	Mean sugar content for entire season	Early season, October–November		Late season, December–February	
		Samples	Mean Sugar content	Samples	Mean Sugar content
		Per cent	Number	Per cent	Number
Ascolano.....	5.54	2	5.48	1	5.66
P. I. G. 27172.....	4.89			2	4.89
Barouni.....	4.59	1	3.56	4	4.84
Sevillano.....	4.09	3	4.87	2	2.92
Mission.....	3.68	4	3.62	4	3.74
Chitoni.....	3.57	1	3.22	2	3.75
Manzanillo.....	2.96	2	2.48	2	3.45
Chemlali.....	2.72	1	2.15	2	3.00
Columbella.....	2.55	2	2.20	1	3.25
Nevadillo.....	2.55	3	2.67	4	2.46
Bidh el Hammam.....	2.39	1	2.04	2	2.56
Saiali Magloub.....	2.06	1	1.48	2	2.35
Uvaria.....	1.92	2	1.72	1	2.30
S. P. I. 27173.....	1.54	1	1.54		
Lucca.....	1.52	1	1.52		
Picholine.....	1.16			1	1.16
Salome.....	1.14	1	1.14		

TABLE 6.—*Total sugars in olives of different varieties expressed in percentage on a dry basis, in the early, late, and entire season*

Variety	Mean sugar content for entire season	Early season, October–November		Late season, December–February	
		Samples	Mean Sugar content	Samples	Mean Sugar content
		Per cent	Number	Per cent	Number
Ascolano.....	18.2	2	19.0	1	16.5
Sevillano.....	16.8	3	21.1	2	10.4
Barouni.....	13.3	1	15.2	4	12.8
P. I. G. 27172.....	10.6			2	10.6
Columbella.....	10.1	2	8.8	1	12.7
Manzanillo.....	9.8	2	9.4	2	10.3
Mission.....	9.5	4	10.6	4	8.4
Chitoni.....	9.0	1	8.2	2	9.0
Uvaria.....	7.3	2	7.6	1	6.7
Bidh el Hammam.....	7.2	1	6.6	3	7.4
Chemlali.....	7.0	1	6.3	2	7.4
Nevadillo.....	6.5	3	10.2	4	6.2
Saiali Magloub.....	6.0	1	4.7	2	6.6
S. P. I. 27173.....	5.3	1	5.3		
Lucca.....	5.3	1	5.3		
Salome.....	4.4	1	4.4		
Picholine.....	3.0			1	3.0

In view of the wide and inconsistent variations shown in Table 4 these means can not be used for close comparisons of varieties. In a relative way, however, the varieties may be divided into three groups with respect to sugar content: Ascolano, P. I. G. 27172, Barouni, and Sevillano, which were high; Mission, Chitoni, Manzanillo, Chemlali, Columbella, Nevadillo, Bidh el Hammam, Saiali Magloub, and Uvaria, which were medium; and S. P. I. 27173, Lucca, Picholine, and Salome, which were low.

SUMMARY

Basic lead acetate was found to be unsatisfactory as a clarifying agent, and to give too high results in the picric acid determination of reducing sugars and sucrose in the water extract of olive flesh.

Reducing substances before and after hydrolysis, remaining in the water extract after clarification by mercuric nitrate were found to be removable by a short treatment with yeast and are believed to be sugars.

The early-season mean ratio of simple to total sugars, expressed as dextrose, usually declined as the season progressed.

The total sugar percentage on the dry basis and in absolute amounts was found to increase in early season and to decrease in late season.

Of the varieties studied Ascolano, P. I. G. 27172, Barouni, and Sevillano were high in sugar content; S. P. I. 27173, Lucca, Picholine, and Salome were low; and the Mission, Chitoni, Manzanillo, Chemlali, Columbella, Nevadillo, Bidh el Hammam, Saiali Magloub, and Uvaria were intermediate.

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EFFECT OF RYE AND VETCH GREEN MANURES ON THE MICROFLORA, NITRATES, AND HYDROGEN-ION CONCENTRATION OF TWO ACID AND NEUTRALIZED SOILS¹

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INTRODUCTION

The depletion of the organic matter and available plant food in soil through certain systems of farming has created a great interest in methods of increasing or even maintaining the fertility of cultivated soils. Animal manure seems to be one of the most effective materials, and its use has always been recommended. However, in this age of motorized transportation manure in large quantities is often difficult to obtain. Aside from the use of animal manure the principal means of augmenting the organic matter in soil is through the use of the so-called green manures. Much work on this subject has been done both in this country and abroad. Most of the literature (33)³ deals with the effect of green manuring upon succeeding crops, the humus content, water-holding capacity, and nitrate content of the soil; the relative rate of decomposition of various plants or parts of plants; the availability of the nitrogen in those plants; or the decomposition of plants of different ages.

Microbiological studies of green manuring, excluding its influence upon nitrification, have not been very numerous or very thorough. Plate counts of soil microorganisms were often made several weeks after the application of the green manure, or the green manure was dried, ground up, thoroughly mixed with the soil, and incubated in small containers. For these reasons there seemed to be a need for closely controlled experiments on the microbiology of decomposing green manures in neutral soils and also in the acid and alkaline soils as found in many of the agricultural districts of the country. Such studies could not be carried out in the field, as they would be subject to climatic variations of temperature and moisture. The alternative seemed to be to transport large quantities of field soil into greenhouses, where those two variables might be controlled. One might say that nowhere in field practice would one find these optimum conditions for any great length of time. This is quite true. However, if what happens under optimum conditions is known it might be of assistance in interpreting results obtained under the variable conditions existing in field experimentations.

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² The authors wish to express their appreciation to Charles Thom, principal mycologist, for his suggestions and interest in the work, and to Daniel Ready, assistant scientific aid, for the nitrate and moisture determinations.

³ Reference is made by number (*italic*) to Literature Cited p. 121.

Since a great many of the eastern soils are naturally acid, it was elected to study the decomposition of green manures in two of these soils which are located fairly close to Washington, D. C., and upon which considerable agronomic and chemical experimental work had been done both by the Maryland Agricultural Experiment Station and the United States Department of Agriculture. The work was begun in the fall of 1927 and finished in the fall of 1928.

MATERIALS AND METHODS

SOILS AND MANURES

The two acid soils selected were of divergent physical character. The fine-textured soil, a Leonardtown clay loam with a pH value of 4.6, was obtained from an uncultivated area on the experimental tract of the Maryland Agricultural Experiment Station located about 3 miles from Leonardtown, Md. The coarser textured soil, a Collington fine sandy loam with a pH value of 5.2, was obtained from an uncultivated area on the experimental tract of the United States Department of Agriculture near Upper Marlboro, Md.

A sufficient amount of each soil was secured to fill two benches in a greenhouse on the Arlington Experiment Farm, Rosslyn, Va. Each bench was divided into 14 sections 3 feet square. Each section had a waterproof cement bottom provided with a drain and wooden sides 12 inches high. The soil after thorough mixing was placed in the sections to a depth of 10 inches and watered with distilled water. It was then kept at the optimum moisture content throughout the experiments.

Sufficient finely ground limestone (100 mesh) was thoroughly mixed with the soil in the even-numbered plots in each series to bring the soil to approximately a neutral reaction. This required a rate of 5 tons per acre for the Leonardtown clay loam and 2.5 tons per acre for the Collington fine sandy loam. (Table 10.) Analysis of the limestone showed an equivalent of 91 per cent CaCO_3 .

The first experiment consisted in turning under green rye as a green manure on a limed and an unlimed plot of each soil. The rye grown on a separate plot in the greenhouse to a height of 15 inches was pulled up by the roots, washed free from soil, and turned under in a layer about 5 inches below the surface of the soil. The rye was therefore in a succulent condition, consisting largely of leafy material. Each plot received 2,200 gm. of green rye, a rate of 23,476 pounds per acre. The air-dry weight of the rye was 25 per cent of the green weight and the nitrogen content was 3.33 per cent. The rate of application was therefore 5,869 pounds of dry material, containing approximately 200 pounds of nitrogen per acre.

Soil samples consisted of a number of cores of soil taken with a sampling tube $1\frac{1}{4}$ inches in diameter to a depth of 6 inches. Samples were taken just before the green material was turned under and subsequently 2, 4, 7, 14, 21, 35, 56, 84, and 119 days after it was turned under. Plate counts of total number of microorganisms, fungi, actinomycetes, and determinations of moisture, of nitrates, and of soil reaction were made of each sample.

In the second experiment vetch was substituted for rye as a green manure on some additional plots of the two soils. It was applied in the same manner as the rye, at the rate of 5,000 gm. of green material.

per plot, equivalent to an application of 53,355 pounds per acre. The dry weight of the vetch was 27 per cent of the weight of the green material and the nitrogen content was 2.5 per cent. This is equivalent to an application of 14,406 pounds of air-dry vetch and 360 pounds of nitrogen per acre.

Analyses were made as in the first experiment, samples being taken in a similar manner 0, 2, 4, 7, 14, 21, 35, and 56 days after the green manure was turned under.

The treatment of the plots may be summarized as follows:

Plot No	Treatment	Plot No	Treatment
1	Control	5	Control
2	Limestone only	6	Limestone only
3	Rye green manure	7	Vetch green manure
4	Rye green manure and limestone.	8	Vetch green manure and limestone

In order to confirm the results obtained on the first two experiments, the green-manure treatments were duplicated on the same plots in the fall of 1928, the plots having been kept fallow all summer at optimum moisture content. Both the rye and the vetch in this experiment were grown on plots equal in size to those treated, but were located in another bench. The amount of green manure applied, therefore, was determined by the yield of rye or vetch produced on these other plots. The amounts applied are shown in Table 1.

TABLE 1 - Amount of green manure and its nitrogen equivalent applied to two arid soils

Plot No	Green manure	Collington fine sandy loam					Leonardtown clay loam				
		Green weight	Equivalent dry weight	Equivalent dry weight per acre	Nitrogen	Nitrogen per acre	Green weight	Equivalent dry weight	Equivalent dry weight per acre	Nitrogen	Nitrogen per acre
		Grams	Grams	Pounds	Per cent	Pounds	Grams	Grams	Pounds	Per cent	Pounds
3	Rye..	2,450	764	8,145	1.93	157	1,020	318	3,390	1.93	65
4	Rye ..	2,590	800	8,530	1.93	166	1,430	446	4,750	1.93	92
7	Vetch ..	1,670	554	5,900	2.04	120	1,140	378	4,000	2.04	82
8	Vetch ..	2,050	680	7,250	2.04	148	1,470	488	5,200	2.04	108

* Limed duplicate

Soil samples were taken 0, 2, 4, 7, 14, 21, 28, 42, and 63 days after the green manure was turned under, and analyses were made as in the first experiment.

Conditions in the greenhouse with the higher temperature and optimum moisture are quite different from those in the field. For this reason it seemed advisable to include a field test along with the first experiment. In this test both rye and vetch were used as green manures. The vetch was from the same lot as that used in the greenhouse and was applied at the same rate. The air-dry weight of the rye used was 20.7 per cent of the green weight and the nitrogen contained was 2.39 per cent. This application was equivalent to 4,860 pounds of dry matter and 116 pounds of nitrogen per acre. The amount of

nitrogen added in this case was considerably lower than that added in the vetch or that in the rye of the first greenhouse experiments. Treatments were as follows:

Plot No.	Treatment	Plot No.	Treatment
1	Rye green manure.	3	Vetch green manure.
2	Control		

The soil in this field experiment was similar in texture to the Leonardtown soil. The pH value was 5.5.

PLATING METHODS

As soon as the soil samples arrived in the laboratory they were put through a 10-mesh sieve and thoroughly mixed. Aliquots were then weighed out for determination of the microflora, nitric nitrogen, and moisture.

Dilutions of the soil samples for plating were made in the following manner. To 20 gm. of soil in a sterile mortar enough sterile tap water was added from the dilution flask to make a thin paste. After triturating until the paste was smooth the remainder of the 500 ml. of sterile water was added, mixed thoroughly, and poured back into the dilution flask. This flask was then stoppered and shaken several times over a period of 10 to 15 minutes. Higher dilutions were made by adding 10 ml. to 90 ml. sterile tap water, the final dilution being 1 to 2,500,000. For the total plate counts, 5 ml. of this dilution were pipetted into each of 10 Petri dishes, 120 by 15 mm. In cases where the number of colonies developing on the plate exceeded 200, only 2.5 ml. were used. The actual dilutions were therefore 1 to 500,000 and 1 to 1,000,000, respectively. For the counts of the actinomycetes, 1 ml. of the 1 to 250,000 dilution was pipetted into each of 10 plates, and for the fungi counts, 5 ml. of the 1 to 25,000 dilution.

The medium used in pouring the plates for the total counts was soil-extract agar, which has been shown to favor the growth of a greater number of soil bacteria than any other commonly used medium (35). The composition of the soil-extract medium is as follows: Soil extract, 1,000 ml.; K_2HPO_4 , 0.5 gm.; and agar, 10 gm. If not pH 6.8, reaction should be adjusted to this. The soil extract is made by autoclaving 1,500 gm. of air-dry field soil of ordinary fertility with 4.5 liters of tap water for one-half hour and filtering clear through paper. The final volume of soil extract is approximately twice the original amount of soil. Large quantities may be prepared, put into large flasks, and sterilized for future use.

The plates for the total counts were incubated for three weeks at 28° C. The results given are the average of 10 plates, the number of organisms per gram of soil being calculated by multiplying the average number per plate by the dilution and correcting the result for the moisture content at the time of sampling.

The medium used in pouring the plates for the actinomycetes consisted of soil extract to which was added 0.1 per cent K_2HPO_4 , 0.1 per cent $NaNO_3$, 1 per cent glycerin, and 1 per cent agar. The reaction was adjusted to pH 6.8. The incubation period for the plates was 10 days at 28° C.

For the estimation of the fungi an acid medium of the following composition was used: Soil extract, 1,000 ml.; KH_2PO_4 , 1 gm.; NaNO_3 , 1 gm.; agar, 25 gm., and dextrose, 10 gm. The dextrose is added after melting and the reaction adjusted to pH 4.2 by adding equal quantities of N HCl and $\text{N H}_2\text{SO}_4$. This usually requires about 1 ml. of each. Care in sterilizing this highly acid medium must be exercised or the agar will be broken down. It has been found safe both as regards the solidifying power and the sterility of the medium to autoclave at 12 pounds pressure for 12 minutes provided the medium is in a water bath during the heating. The plates were incubated for three days at 28°C .

LITERATURE REVIEW

The literature dealing with the effects upon the numbers of bacteria of neutralizing the soil acidity with limestone or other forms of lime is quite voluminous. While no attempt will be made in this paper to cover it, it may be advisable to review briefly some of the work along this line.

Chester (15), Fabricius and Von Feilitzen (18), and Fischer (19) were among the first to note that lime increased the number of bacteria. Brown (10, 11) found that lime increased the plate counts of soil in pots in the greenhouses and also those of field soils. Beckwith, Vass, and Robinson (6) studied the effect of lime on soils of varying reaction and found that lime increased the number of bacteria only in the acid soils. In one acid soil high in organic matter, while increasing the number of bacteria, lime decreased the number of molds.

Bear (5), applying various amounts of CaCO_3 to soil, reported increases in the number of bacteria up to the heaviest application (40,000 pounds) 12 weeks after treatment. Fulmer (21) found that calcium carbonate, magnesium carbonate, and limestone increased the bacteria in two acid soils, magnesium carbonate producing the greatest effect. Noyes and Connor (30) found that calcium carbonate increased the bacterial contents of 5 typical acid soils 10 months after application in the greenhouse and that the increase was due to aerobic bacteria.

Waksman (38) found that lime added to an acid sandy loam not very rich in organic matter increased the bacteria and actinomycetes and decreased the fungi.

Barnette (4) used limestone and other forms of calcium and found a rapid increase in total plate counts for two weeks, which was followed by a decrease to nearly a constant level at four weeks. Chatterjee (14) also reported an increase in total counts due to liming.

The effect of turning under green manures upon the total number of microorganisms has been studied to a less extent. Engberding (17) added both green rye and vetch to soil and found that the plate counts of the microorganisms had increased at the end of 7 weeks from 17,000,000 to 100,000,000 with the rye and to 80,000,000 with the vetch. After 10 weeks they had dropped to 78,000,000 and 60,000,000, respectively, and the checks had increased to 30,000,000. Brown (12), on the other hand, reported that green manuring with rye, cowpeas, and clover did not always increase the number of bacteria in field soil. This may have been partly due to the low moisture content of the soil. In a 2-year rotation (13), turning under rye increased the plate counts over those of the regular rotations.

Briscoe and Harned (7, 8), using green alfalfa, oats, and cowpeas for green manure, found a direct relationship between the total plate counts of soil microorganisms and the amount of organic matter added. How long the materials were allowed to decompose before the samples were taken was not stated. Furthermore, different kinds of plating media were used during the experiments.

Hill (23) obtained a great increase in the number of bacteria in various soils treated with bluegrass, clover, and alfalfa green manures. Clover, especially, increased the numbers at four weeks, the numbers decreasing after that.

Baldwin and Smith (3) applied green rye at the rate of 12 tons per acre and found after one month an increase in the number of bacteria growing upon beef agar. After three months the treated soil contained approximately the same number as the controls.

Martin (27) added rye, oats, and buckwheat as green manure and allowed it to decompose 12 months before sampling. Realizing

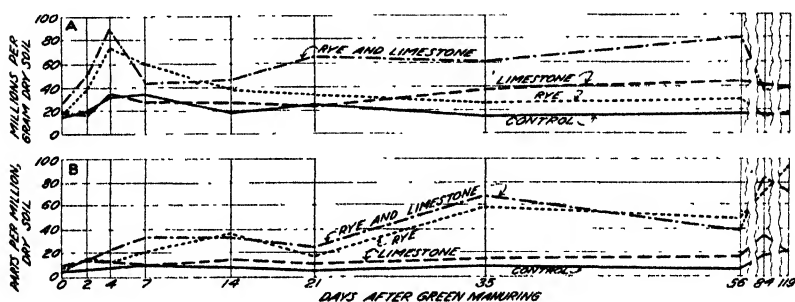


FIGURE 1 - Comparison of total plate counts (A) and nitrate nitrogen (B) in Collington fine sandy loam as affected by different treatments; rye green-manure series

that the interval was too long, he ran another series and took samples at the end of 4 months. In this series no control was analyzed. In general, total counts were higher in the green-manured soil.

EFFECT OF TREATMENTS ON THE TOTAL PLATE COUNTS OF THE SOIL MICROFLORA

It will be recalled that there were three treatments for each acid soil and each green manure, viz—(1) limestone, (2) rye or vetch, and (3) limestone and rye or vetch. Also certain conditions of the experiment should be emphasized, viz, the application of the whole green plant (roots and tops), the uniform depth of 5 inches at which the green material was turned under, the constant temperature and moisture, and the frequent samplings during the early stages of decomposition of the green manure.

The total counts made on the four plots of the Collington fine sandy loam of the rye green-manuring series are shown in Figure 1. The untreated control shows some variation in numbers during the course of the experiment, but as a whole the counts are fairly uniform. The numbers in the limed plot followed those in the control until the thirty-fifth day, when there was an increase to nearly 40,000,000, this figure persisting to the end.

When the green rye was turned under a rapid increase in the number of microorganisms took place both on the limed and on the unlimed plots. In 4 days the number increased to 74,000,000 where rye alone was added and to 90,000,000 with rye and limestone. A rapid decrease followed, which in the latter case was followed by another increase, reaching a peak at 80,000,000 in 56 days. The acid rye-manured plot, on the other hand, shows a gradual decrease in total counts from the fourth to the thirty-fifth day, the numbers remaining only slightly above the control until the end of the experiment, when they were practically identical. At the end the plate counts of the two limed plots reached the same level in spite of the addition of rye to one of them, and this level was well above that of the other two plots which had no limestone.

The results of plating out the Leonardtown series are given in Figure 2. The control, as in the Collington series, shows only a

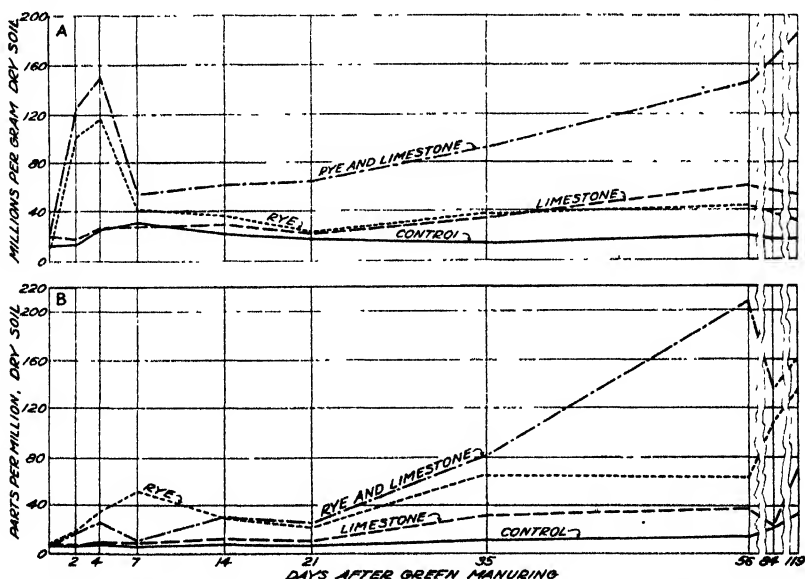


FIGURE 2.— Comparison of total plate counts (A) and nitrate nitrogen (B) in Leonardtown clay loam as affected by different treatments; rye green-manure series

slight change in the number of microorganisms. The limed control shows an increase at the 35-day sampling and a further increase to 60,000,000 in 56 days, the count falling off some at the end. The application of green rye caused an increase to 116,000,000 in 4 days, whereas rye and limestone together gave a peak of 151,000,000 at that time. In both cases there was a sharp drop at the 7-day period to 41,000,000 and 56,000,000, respectively. Thereafter very little change took place in the acid rye-manured plot. But with the rye and limestone together there was a steady rise in the plate counts to the fifty-sixth-day sampling and then a jump to 184,000,000 at 119 days.

In the next experiment the effect of turning under green vetch was studied under similar conditions, with the exception of an irregularity in limestone treatment, which will be discussed later.

Figure 3 shows the relative number of microorganisms found in the four plots of Collington soil of the vetch green-manure series. In the control and limed plots the numbers are similar to those found in the rye series. Vetch in the acid soil affected the organisms much the same as the rye, except that the peak was not reached until the seventh day. In the case of the vetch and limestone the great increase

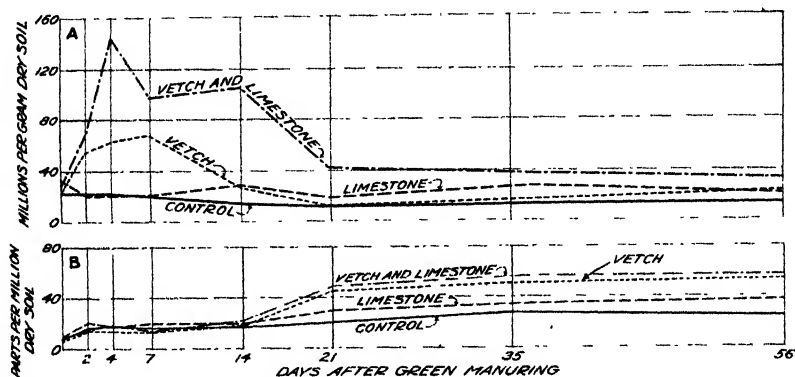


FIGURE 3.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Collington fine sandy loam as affected by different treatments, vetch green-manure series

occurred at four days. At seven days there was a considerable decrease but not until the twenty-first day was it pronounced. There was no secondary increase as found in the rye series.

Plate counts of the four plots of Leonardtown clay loam of the vetch green-manuring series are shown in Figure 4. Here again

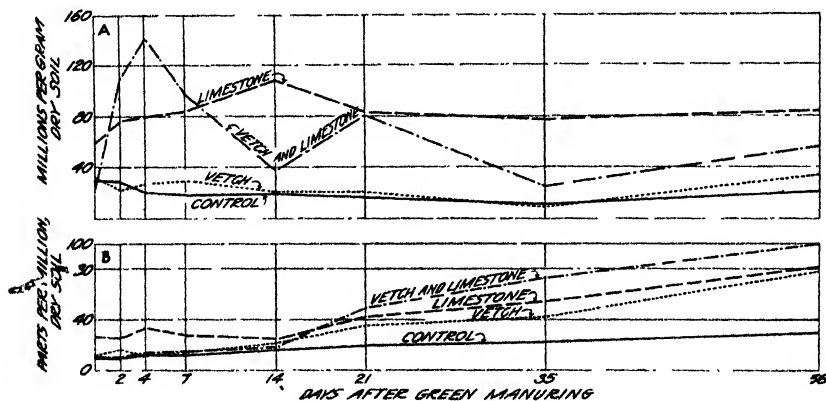


FIGURE 4.—Comparison of total plate count (A) and nitrate nitrogen (B) in Leonardtown clay loam as affected by different treatments, vetch green-manure series

the numbers in the control are fairly consistent. Where limestone and vetch were added there is the same rapid increase in numbers in four days, followed by a drop to relatively near the level at the start. Further comparison with Figure 2 reveals only differences which seem to be inexplicable. For instance, the addition of vetch to the acid soil failed to affect the total counts at any time, the

counts remaining very close to those of the control plot. The high numbers found in the limed plot during the whole period may be partially explained by the fact that the limestone was added to this soil 10 weeks before it was added to the plot receiving the vetch. Dorsey (16) has shown that limestone reduces the acidity gradually for 6 to 8 weeks. Karraker (26) found that the effect of limestone in bringing about an increased growth of alfalfa on acid soils was delayed from a few weeks to two months. This delayed action of limestone may be the cause of the large numbers in the limed plot. However, the corresponding plot of the Collington soil which was also limed

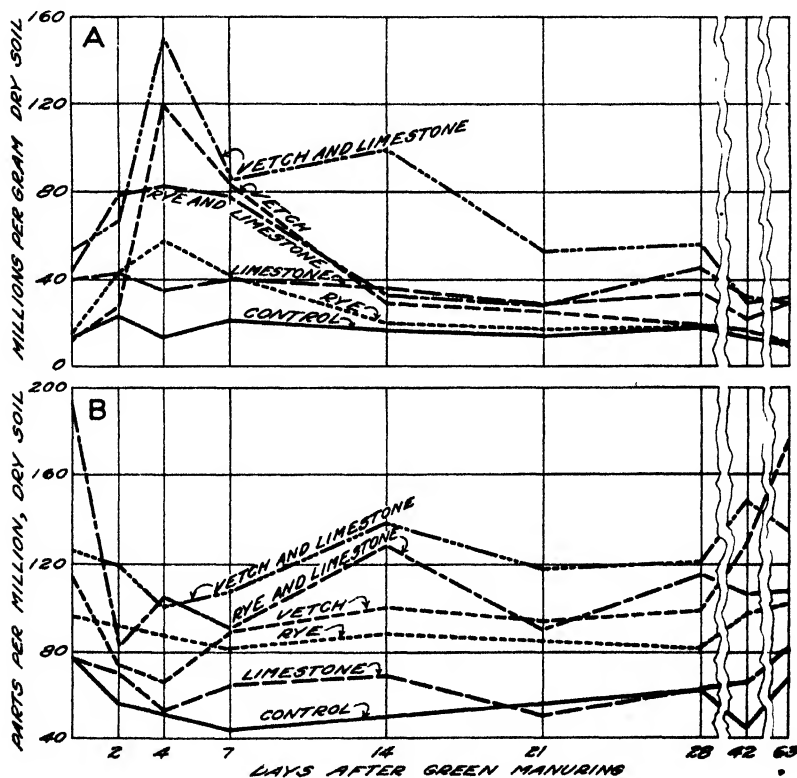


FIGURE 5.—Comparison of total plate count (A) and nitrate nitrogen (B) in Collington fine sandy loam as affected by different treatments, second application of rye and vetch green manures

previously did not show this delayed effect, nor did it show the great variations as noted on the Leonardtown limed plot at 4, 7, and 14 days. Of course, these soils are very different physically, and the Leonardtown is more acid. However, Barnette (4) observed a rapid increase in plate counts for two weeks due to limestone, after which there was a decline to a constant level at four weeks. Whatever the explanation, the fact remains that the limed control plot contained the greatest number of microorganisms after the seventh-day sampling.

For several reasons it seemed advisable to repeat the green-manure treatment on these same plots. This was done in the fall of 1928, the soil in the meantime being kept moist and fallow.

The results on the Collington fine sandy loam are plotted in Figure 5. The plate counts of the three acid plots were the same at the beginning. On the three limed plots, however, they were more than double, the vetch plot highest, rye next, and limed plot lowest. Comparing the end of Figures 1 and 3 with the beginning of Figure 5, it will be noted that the long fallow period produced practically no

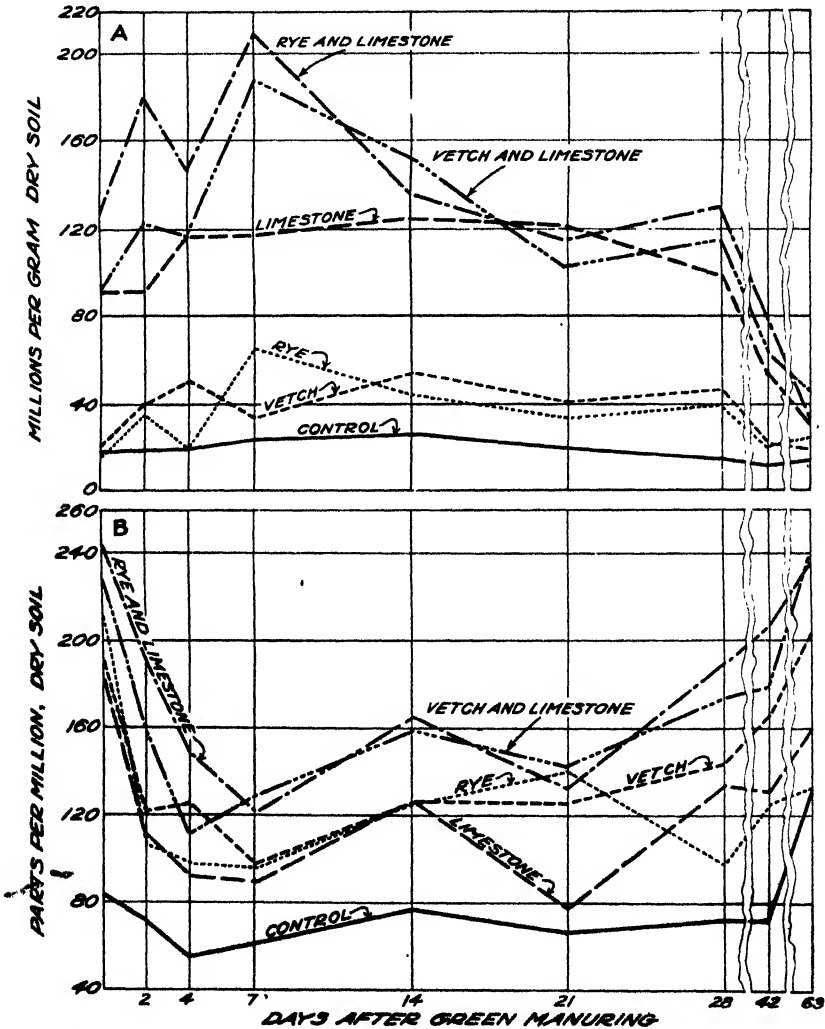


FIGURE 6.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Leonardtown clay loam as affected by different treatments; second application of rye and vetch green manure

change in the total count of the acid plots and only small increases in that of the limed rye and limed vetch plots. Throughout this experiment the plate counts of the control and the limed plots remained fairly uniform. The decomposition of the green manure was exceedingly rapid. The numbers in all cases were very high in four days, especially where vetch was turned under. In both the rye and vetch

acid plots the numbers decreased to the level of the control in 28 days whereas in the limed plots at this time the effect of the green manure was still noticeable, as indicated by a slight secondary rise. Again comparing this figure with Figures 1 and 5, it will be seen that the curves are remarkably similar in spite of the previous treatments.

On the Leonardtown clay loam the effect of the treatments on the number of microorganisms is shown in Figure 6. The addition of green rye to the acid plot caused an increase in number which reached a high point in seven days, a low number, for some unknown reason, being recorded on the fourth day. The usual decrease followed. With vetch turned under, the number of microorganisms increased to 50,000,000 in 4 days, followed by a decrease at 7 days and a subsequent increase to 53,000,000 in 14 days. This was followed by a slight decrease at the next two samplings and quite a definite decrease on the last two sampling dates.

The curves indicating the numbers on the limed plots form a distinct group from the curves of the corresponding unlimed plots.

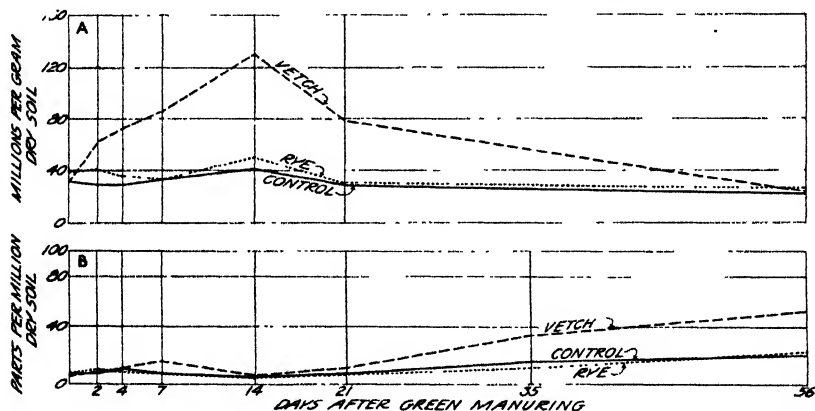


FIGURE 7 - Comparison of total plate counts (A) and nitrate nitrogen (B) in Keysport clay loam as affected by rye and vetch green manuring under field conditions

With limestone alone there were increases for 14 days followed by a slight decrease, which became much larger at 42 and 63 days. The rise in numbers may have been due in part to stirring the soil. All plots were stirred at the same time so as to eliminate any discrepancy which might arise because of the stirring incident to the turning under of the rye and vetch green manures. The limed rye and the limed vetch plots showed the usual rapid increase in numbers to the peak at 7 days, followed by a subsequent decrease. At 63 days the counts of all the limed plots had decreased greatly and were only slightly above those of the unlimed series.

Since the conditions of temperature and moisture were quite different in the greenhouse from what is usually found in the field, a preliminary experiment was made in the field to determine to what extent greenhouse and field results would correlate. The soil for this field experiment was a Keysport clay loam located on Arlington Farm, Virginia. It had been limed some years previously but was slightly acid (pH 5.5).

Both rye and vetch were used as green manures. The total plate counts in the vetch-manured plot (fig. 7) show the characteristic rapid

rise, but instead of a 4-day or 7-day peak the highest count came on the fourteenth day after treatment. The temperature of this field soil was not taken. It is known, however, that it was considerably below that of the soil in the greenhouse which was kept between 20° and 22° C. Moisture was also somewhat lower. These factors undoubtedly slowed up the decomposition and account for the retarded maximum number of organisms.

The rye was field grown and of a slightly different composition than that used in the greenhouse. It affected the number of soil organisms very little, comparatively. No explanation is offered for the failure of the rye to produce an increase in the soil flora in this case.

EFFECT OF TREATMENTS ON THE BACTERIA

The total plate counts of the microorganisms in soil comprise the bacteria, actinomycetes, and fungi. The bacteria are the most numerous ordinarily. In fact, many workers have considered the total plate count as representing the bacteria and have often reported the plate counts as numbers of bacteria. In this paper the authors have preferred to report the total plate counts as such and to report separately the numbers and kinds of bacteria occurring on those plates as determined by cultural and staining methods.

One of the 10 plates of each soil sample used in making the total counts was selected after the counts had been made and all bacterial colonies picked off and inoculated into a nutrient soil-extract agar.⁴ This medium gave a much better growth than the soil-extract agar used for plating and was also better than ordinary nutrient agar for many of the soil organisms. After growth had occurred smears were made and stained by Gram's method as modified by Jensen, reported by Orla-Jensen (32, p. 25, footnote). This method has been used by the senior author for 10 years, and while it gives good results no special recommendation is made for it. Although it is well known that bacteria may vary in their reaction to the Gram stain from time to time, this seemed to be the only feasible method of roughly dividing the bacteria into groups.

In order to control the accuracy of the staining method, one set of 63 bacterial cultures was transferred after the smears were made for the Gram staining. These new cultures were subsequently smeared and stained and were found to have the same reactions except in three cases. This is not considered a serious error.

In Figure 8 are shown the numbers of Gram-negative and Gram-positive bacteria found in the Collington soil of the rye green-manuring series. At first there was a tendency for the members of both groups to increase in number. Later, without limestone, both the negative and positive forms decreased and were no more numerous at 21 days than the control. Where rye was added to the limed soil there was a decrease at 7 days, which was followed by increases in both groups. The Gram-negative forms increased much more than the positive forms and were still high in number at 56 days, whereas the positive forms had decreased to the level of the control. Limestone increased the Gram-negative bacteria, the increase beginning at 14 days and

⁴ The formula for this medium is as follows: Soil extract, 1,000 ml.; K_2HPO_4 , 0.5 gm.; ammonium citrate, 0.5 gm.; peptone, 1.0 gm.; beef extract, 0.5 gm.; $NaNO_3$, 0.5 gm.; $MgSO_4$, 0.2 gm.; $CaCl_2$, 0.2 gm.; dextrose, 5 gm.; and agar, 10 gm. Reaction adjusted to pH 6.8 by NaOH.

rising steadily to the end. It produced no effect on the positive group.

The Gram-negative and Gram-positive bacteria in the more acid Leonardtown clay loam (fig. 9) were affected to a greater degree than they were in the Collington soil. The initial increase of both

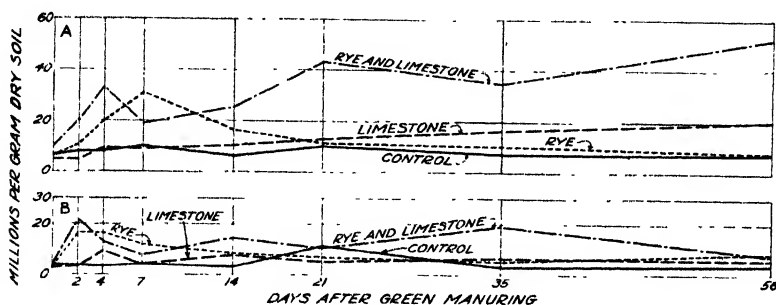


FIGURE 8.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Collington fine sandy loam, as affected by different treatments, rye green-manure series.

kinds was very marked, rye alone apparently producing the greatest stimulation of the positive group, whereas rye and limestone most strongly affected the negative group. The sudden fall in the number of both at the 7-day period is very striking and agrees with the fall in the total counts. (Fig. 2.) The positive group increased again somewhat in the case of rye and limestone, but it was not

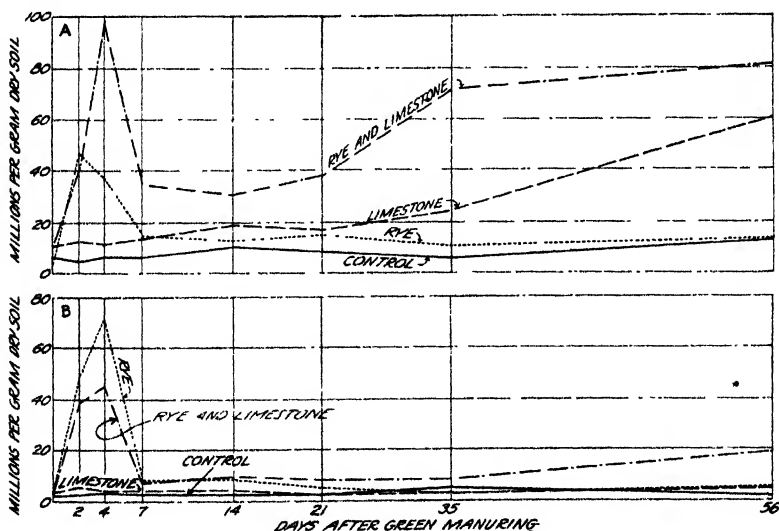


FIGURE 9.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Leonardtown clay loam, as affected by different treatments, rye green-manure series.

affected by either the rye or limestone when used separately. On the other hand, the Gram-negative group increased enormously in the limed rye-treated plot and also in the limed plot. In the latter case the effect of the limestone was delayed until the thirty-fifth-day sampling. Comparing this figure again with Figure 2, it is very

evident that the secondary increase in the total plate counts is due

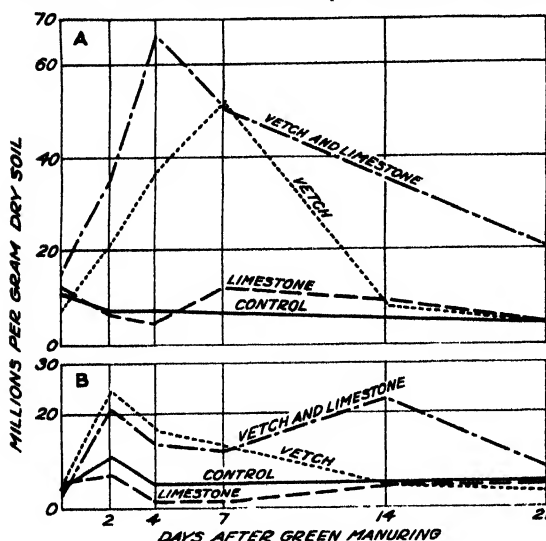


FIGURE 10.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Collington fine sandy loam, as affected by different treatments, vetch green-manure series

to the increase in the bacteria, and that this increase is, to a large extent, due to the effect of the limestone upon the Gram-negative forms.

In the vetch-manuring experiment on the Collington soil the Gram-negative group (fig. 10) increased much faster and to a greater height than it did when rye was added (fig. 8). With vetch alone the highest number occurred on the seventh day. At 21 days the counts had decreased to those of the control and limed plots. As mentioned above, this

limestone plot is not comparable to the one in the rye green-manure series, due to the fact that 10 weeks elapsed between the application of the limestone and the turning under of the vetch. Vetch and limestone increased the negative group to over 66,000,000 in 4 days. Then a sharp decline to 20,000,000 occurred at 21 days.

The Gram-positive group increased 2 days after the vetch was turned under, but the fourth and seventh day samplings showed a decrease. After 14 days this group was no more numerous than the controls. In the limestone-vetch soil there were two maxima (2 and 14 days). The numbers were reduced to practically the same level as the controls at 21 days.

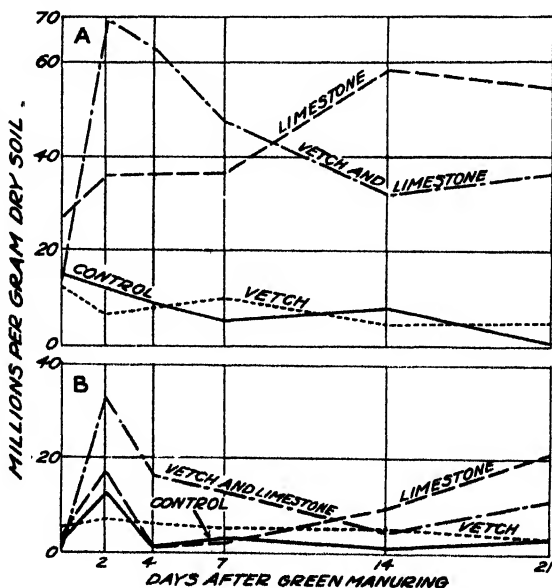


FIGURE 11.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Leonardtown clay loam, as affected by different treatments, vetch green-manure series

The numbers were reduced to practically the same level as the controls at 21 days.

The isolation of cultures was stopped with the twenty-first-day sampling because the total plate counts showed no secondary increase as in the first experiment and only a slight decrease after this period.

The turning under of vetch in the Leonardtown soil did not have the same effect as in the Collington, nor was the effect similar to that produced by rye in either soil. This fact was noted in dealing with the total counts and no explanation can be offered for it. For instance, the turning under of vetch in the unlimed soil had no effect upon either the Gram-negative or the Gram-positive bacteria. (Fig. 11.) However, on the limed soil vetch increased both groups at two days as in the other experiments. Thereafter, while these two groups were decreasing on the vetch-limestone plot, they were increasing under the effect of limestone alone.

The effect of rye and vetch green manures upon the Gram-negative and Gram-positive bacteria in Keysport clay loam under field conditions is shown in Figure 12. Both groups of bacteria seem to be equally affected, the highest counts occurring with both green manures at the 14-day sampling. The vetch, as stated above, was from the same lot that was turned under in the greenhouse soil, whereas the rye was field grown and less succulent.

This may have had some influence on the development of the bacteria. At least, this figure shows that the increase in the bacteria is slowed up under field conditions but that there is rapid rise in numbers during the first two weeks of the decomposition of vetch. The decomposition of the rye caused only a slight increase at the 14-day sampling.

The isolation of cultures was stopped with the 21-day sampling. It is not likely that any radical change would have been found at later samplings, for the total counts (fig. 7) continued to decrease and were on a level with the control at 56 days.

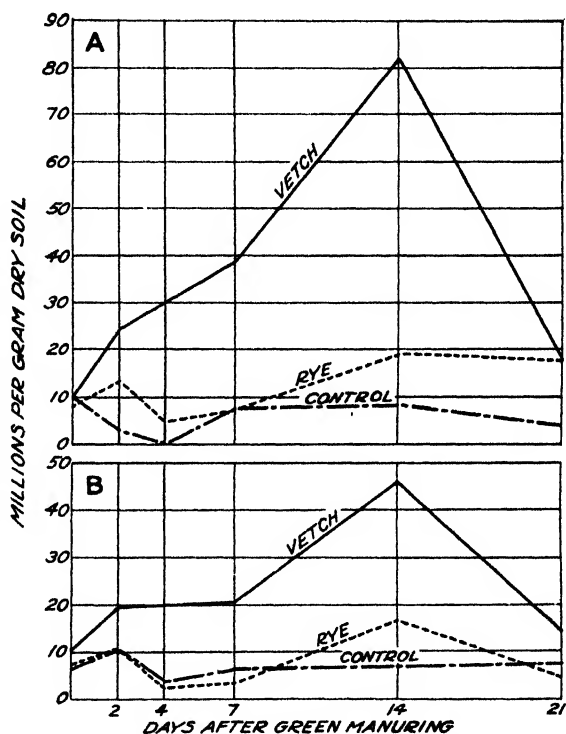


FIGURE 12 - Number of Gram-negative (A) and Gram-positive (B) bacteria in Keysport clay loam, as affected by rye and vetch green manures

EFFECT OF TREATMENTS ON ACTINOMYCETES

The number of actinomycetes present in the soil samples were determined as described above. The counts on the Collington and Leonardtown soils of the rye green-manuring series are given in Table 2.

TABLE 2.—*Millions of actinomycetes per gram of dry soil in two acid soils in the rye green-manure series when limed and unlimed*

Days after green manuring	Collington fine sandy loam				Leonardtown clay loam			
	Control	Limed control	Rye	Limed rye	Control	Limed control	Rye	Limed rye
0.....	2.6	3.0	3.2	4.0	2.4	2.6	3.0	2.9
2.....	2.7	3.3	4.4	3.5	3.4	4.2	3.0	3.0
4.....	3.4	4.3	3.4	4.5	3.4	3.0	3.2	2.6
7.....	2.9	3.1	2.4	2.4	3.4	—	3.7	2.3
14.....	2.7	3.6	2.8	3.4	3.6	3.9	3.0	4.2
21.....	3.1	3.8	3.5	4.8	3.4	3.2	3.9	4.5
35.....	2.2	3.0	3.7	4.1	2.5	1.9	3.5	2.8
50.....	2.0	3.4	3.6	5.5	3.8	3.5	3.5	2.9
84.....	1.4	2.0	1.3	1.9	2.3	2.2	2.2	2.1

No significant increase in the number of actinomycetes occurred in the Leonardtown soil when limestone, rye green manure, or both were added. In the Collington soil there seems to have been a slight increase due to the treatments. On account of the great variations in the counts from one sampling to another, no importance should be attached to these small increases.

In the second experiment vetch was turned under in place of rye. The number of actinomycetes (Table 3) decreased slightly where vetch was added and rose slightly with limestone alone. This holds true for both soils. The decrease in the number of actinomycetes took place during the first seven days coincident with the highest counts of the bacteria. Millard and Taylor (28) found that a saprophytic actinomycete competed with a pathogenic form for the available food in green-manure experiments, and suggested that bacteria might also compete with weaker forms and inhibit their development. The bacteria may have inhibited the actinomycetes in the experiment here recorded. On the other hand, the actinomycete plates were heavily seeded with bacteria of the type producing an abundant growth. Therefore there may have been competition on the plates, with the result that a large number of the actinomycetes were either inhibited or so masked that the colonies could not be differentiated. The latter explanation seems the more plausible, especially since subsequent samplings showed, on the whole, the same number of actinomycetes as at the start.

TABLE 3.—*Millions of actinomycetes per gram of dry soil in two acid soils in the vetch green-manure series when limed and unlimed*

Days after green manuring	Collington fine sandy loam				Leonardtown clay loam			
	Control	Limed control	Vetch	Limed vetch	Control	Limed control	Vetch	Limed vetch
0	2.6	2.9	2.8	2.7				
2	3.5	3.3	2.0	1.3	3.0	4.1	2.2	1.5
4	3.0	3.0	1.6	1.4	3.1	2.8	1.2	2.2
7		2.4	.8	.7	1.9	1.8	.9	.6
14	1.5	3.5	2.2	2.3	1.9	2.2	1.9	2.0
21	2.2	2.4	1.9	2.5	2.1	2.8	2.4	1.9
35	1.5	1.8	2.1	2.4	2.1	2.6	1.7	1.8
50	2.1	2.0	2.8	2.7	2.6	2.7	2.6	2.8

As stated above, green rye and green vetch were again added to the same plots after several months of fallow under moist and rather hot conditions. Table 4 shows the counts of actinomycetes obtained. Here, again, there were lower counts in the fourth and seventh day samplings. The rye and vetch green manures on the acid plots of both soils seem to have inhibited the number of actinomycetes more than where limestone was also applied. This effect was not observed in the first application of the green manures. These results are rather surprising, for the reason that the total plate counts, especially of the limed green-manure plots of the Leonardtown soil, were at least three times those of the acid plots. In the Collington soil under the same conditions there was not quite so much difference between the limed and acid green-manure plots. With this soil, though, there was quite a difference in the plate counts of the acid vetch and acid rye plots. The actinomycetes in both cases, however, were the same. It seems, therefore, that it is not so much the number as it is the kind of bacteria which inhibits the development of the actinomycetes on the plates. The bacteria grew rapidly on the glycerin-nitrate agar and oftentimes spread over half the plate.

TABLE 4.—*Millions of actinomycetes per gram in two acid soils, limed and unlimed, after the second application of rye and vetch green manures*

Days after green manuring	Collington fine sandy loam						Leonardtown clay loam					
	Control	Limed control	Rye	Limed rye	Vetch	Limed Vetch	Control	Limed control	Rye	Limed rye	Vetch	Limed Vetch
0	2.4	2.3	1.4	1.7	1.7	2.7	2.4	3.7	1.9	2.4	2.2	2.2
2	3.1	3.0	2.2	3.0	2.7	2.3	2.5	2.8	2.8	3.1	2.9	3.8
4	2.4	2.2	1.4	2.2	1.4	1.9	2.3	1.7	1.1	2.0	1.8	2.0
7	2.7	2.9	1.5	2.9	1.6	3.4	3.2	5.0	2.0	2.0	1.4	2.4
14	2.8	2.5	2.2	2.9	2.8	4.8	3.3	3.5	2.9	2.6	3.3	3.2
21	2.8	3.1	2.0	3.1	3.0	4.0	3.3	4.0	3.0	3.1	3.1	3.3
28	3.6	4.1	3.3	3.8	2.4	3.9	4.2	4.2	3.6	3.4	4.1	3.2
42	2.1	1.9	1.4	1.5	1.4	1.7	1.4	1.7	1.6	2.2	1.5	2.2
63	1.6	2.8	2.1	1.4	.6	1.8	2.7	1.9	2.7	3.1	2.0	3.0

The numbers of actinomycetes in the rye and vetch green-manure plots of Keysport clay loam are given in Table 5.

Although the numbers in this soil were about double those in the Collington and Leonardtown soils, no greater uniformity of counts was obtained; in fact, the fluctuations seem to be worse. Vetch caused

a decrease in the actinomycetes, probably by increasing the numbers of bacteria, some of which, as noted above, grew well on the medium. This is in harmony with the results of the other experiments. Rye, as noted above, failed to produce an increase in the bacteria in this soil. Therefore there was no crowding of the actinomycetes on the plates and the counts averaged the same as the control.

TABLE 5. *Millions of actinomycetes per gram of dry soil in the field experiment on Keysport clay loam, rye and vetch green manures being used*

Days after green manuring	Rye	Control	Vetch	Days after green manuring	Rye	Control	Vetch
0. ---	8 7	6 9	5 7	14 ---	3 8	5 8	1 8
2 ---	7 5	7 4	5 0	21 ---	3 6	5 4	1 5
4 ---	7 6	7 8	7 7	35 ---	3 8	6 4	6 5
7. ---	4 4	4 8	1 3	56 ---	4 3	3 9	4 5

EFFECT OF TREATMENTS ON FUNGI

For the determination of the number of fungi, plates were poured as described above and the colonies counted after three days. In Table 6 the counts on both the Collington and Leonardtown soils of the rye green-manure series are given. In both soils there was a slight increase in fungi on the second and fourth day samplings, especially on the unlimed Leonardtown soil. The increase was of short duration, for at 7 days the counts for the most part had returned to normal. At 14 days they seem to have decreased below the average. On both soils the highest counts of fungi were obtained on the unlimed soil, as might be expected. However, the increase in the fungi was really insignificant compared with the increase in the bacteria. The decomposition was, then, bacterial, and while the fungi did increase somewhat at first, they could not compete with the bacteria under these conditions.

TABLE 6.—*Thousands of fungi per gram of dry soil in two acid soils in the rye green-manure series, when limed and unlimed*

Days after green manuring	Collington fine sandy loam				Leonardtown clay loam			
	Control	Limed control	Rye	Limed rye	Control	Limed control	Rye	Limed rye
0	105	109	117	149	207	209	194	188
2	98	130	168	132	221	240	344	317
4	170	203	230	201	291	292	649	405
7	91	118	180	140	225	205	200	214
14	77	69	81	65	177	141	124	145
21	90	90	77	96	201	192	168	211
35	62	110	79	89	148	86	140	145
56	121	110	95	125	206	187	166	168
84	55	99	112	89	214	210	240	163

Vetch green manuring on these soils had even less effect than the rye upon the fungi, as shown by the results in Table 7. All the soils gave slightly lower fungi counts than when rye was turned under, but that is immaterial since the controls in this experiment were as uniform as in the former.

In this experiment, limestone seems to have increased the number of fungi somewhat in the Collington soil, whereas in the Leonardtown

soil it had the opposite effect. The effect in either case was slight if it be compared with that produced by the limestone upon the bacteria.

TABLE 7.—Thousands of fungi per gram of dry soil in two acid soils in the vetch green-manure series when limed and unlimed

Days after green manuring	Collington fine sandy loam				Leonardtown clay loam			
	Control	Limed control	Vetch	Limed vetch	Control	Limed control	Vetch	Limed vetch
0.	74	100	108	85	169	115	170	122
2	102	126	96	102	188	191	117	222
4	98	98	100	99	149	154	75	98
7	75	104	103	162	177	182	192	185
14	85	97	91	106	199	167	140	168
21	99	89	122	117	131	144	196	164
35	100	87	77	87	142	140	198	128
49	97	96	93	102	184	180	198	178

The second application of rye and vetch green manures to those plots after a long fallow period had less effect upon the number of fungi than did the first application, as is shown in Table 8. It will be noted that the fungi had increased in the controls and also in the previously treated Leonardtown soil during the fallow period when no samples were taken. These increases in the number of fungi are not very large as compared with the increases observed in the total plate counts. The fact that the fungi failed to develop, especially in the acid green-manure plots, was very surprising and was one of the chief reasons for repeating the applications of green manures on these plots.

TABLE 8.—Thousands of fungi per gram of dry soil in two acid soils, limed and unlimed, after the second application of rye and vetch green manures

Days after green manuring	Collington fine sandy loam						Leonardtown clay loam					
	Control	Limed control	Rye	Limed rye	Vetch	Limed vetch	Control	Limed control	Rye	Limed rye	Vetch	Limed vetch
0	114	122	113	105	129	109	248	295	312	312	232	302
2	140	135	153	126	126	141	261	226	353	355	323	329
4	114	90	142	138	128	128	219	238	280	350	291	219
7	116	97	135	175	175	172	251	339	388	374	294	273
14	106	123	123	141	165	169	264	208	377	358	397	263
21	83	95	119	105	149	138	211	270	316	345	351	272
28	121	99	147	125	152	117	235	242	299	288	286	187
42	126	93	129	132	147	149	206	247	256	380	335	202
63	111	109	123	97	154	134	176	253	233	240	300	258

The effect of rye and vetch green manuring on the fungi in Keysport clay loam under field conditions is shown in Table 9.

TABLE 9.—Thousands of fungi per gram of dry soil in the field experiment on Keysport clay loam, rye and vetch green manures being used

Days after green manuring	Rye	Control	Vetch	Days after green manuring	Rye	Control	Vetch
0.	138	159	150	14.	178	190	200
2.	137	152	180	21.	147	153	192
4.	163	179	126	35.	152	178	196
7.	165	196	180	56.	165	151	198

Here, again, no increase in fungi is discernible. On the other hand, the number on the rye-treated plot is slightly smaller than on the control and vetch plots. This is just the reverse of what was expected. It had been thought that the less succulent condition of the rye green manure and the drier condition of the soil as compared with that in greenhouse experiments would stimulate the fungi.

No definite increase took place in the number of fungi at any time. These results are not in accord with those found by Waksman and Starkey (39). They reported that green manure increased both bacteria and fungi and that "the number of fungi increased more abundantly in the acid soils than in those more nearly neutral in reaction." Alfalfa meal was used as the green manure and mixed with soil in tumblers. Neller (29) decided that the fungi were the most active of the soil flora. He based his claim on the fact that pure cultures of these produced the same effects as a soil infusion when alfalfa meal was mixed with sandy soil in tumblers. The conditions of these experiments and those reported in this paper are so different it is no wonder that the results do not correlate. In studying the effects of green manure it would seem advisable to use the material in its natural condition and to incorporate it into the soil in such a manner that field conditions might be more nearly reproduced.

EFFECT OF TREATMENTS ON THE ACCUMULATION OF NITRATES

In connection with the determination of the number of the bacteria, actinomycetes, and fungi, the amount of nitrogen as nitrates was determined on each soil sample. One hundred grams were weighed out and placed in a flask. To the Collington samples, 450 ml. of distilled water and 50 ml. of a 10 per cent suspension of aluminum cream, and to the Leonardtown samples, 400 ml. of distilled water and 100 ml. of the cream were added. The suspensions were shaken intermittently for an hour and filtered. Two 100 ml. aliquots were evaporated on the steam bath and the nitrates determined by the phenoldisulphonic-acid method. Correction for moisture was made and the results reported as parts per million. If the soil contained more than 50 parts per million of nitrate-nitrogen, small aliquots were taken.

The nitrates in the first rye green-manure experiment on Collington fine sandy loam are shown in the lower part of Figure 1. There was a slow increase in nitrate nitrogen in the control plot, as might be expected of field soil which was brought into the greenhouse and kept fallow under optimum conditions.

In the limed soil the nitrates were somewhat higher throughout the experiment, indicating only a slight effect of limestone on the nitrification of the soil nitrogen.

In the rye green-manure plots (fig. 1), with or without limestone, there was a large increase in nitrates within 7 days. While there was somewhat of a drop at 21 days, the nitrates at 35 days had increased on an average to more than 60 parts per million and remained high thereafter. It is interesting to note that although the pH value of the original soil was 5.2, limestone apparently made no difference in the nitrification of the nitrogen of the rye green manure.

Analogous results were obtained on Leonardtown clay loam with rye green manure. (Fig. 2.) The drop in nitrates at the seventh-day sampling of the rye-limestone plot is inexplicable. Since the other data on the limed and acid rye plots are consistent, it may be assumed that this seventh-day determination was wrong. After 35 days the nitrification of the rye green-manure nitrogen was slowed up in the acid soil, whereas in the limed the nitrates rose to over 200 parts per million in 56 days. However, at 119 days the acid plot was nearly as high as the limed.

Vetch green manure on the Collington soil, either limed or unlimed (fig. 3), gave results similar to the rye, except that nitrification was slower. The nitrates in all the plots were practically the same up to the 21-day sampling. Then the soils receiving the application of vetch green manure definitely contained more nitrate nitrogen. This condition persisted to the end of the experiment at 56 days.

On the Leonardtown soil (fig. 4), vetch green manure gave erratic results. It will be remembered that the total plate counts on these plots were not in accord with the other experiments, especially in regard to the behavior of the acid vetch plot. The nitrates in the control soil increased slowly and those in the limed soil more rapidly. The vetch-limestone plot showed the largest increase, reaching 100 parts per million at 56 days. The limed control soil at this period contained about 80 parts per million of nitric nitrogen.

When the second applications of rye and vetch were made on the respective plots of the Collington soil six months after the first, the nitric nitrogen had increased considerably in all plots. The limed green-manure plots had the largest amount, the acid rye and vetch the next largest, and the limed and control plots the smallest. (Fig. 5.) In two days after green manuring there was a decrease in all cases. This was partly due, perhaps, to stirring the soil, since the two controls also showed the decrease, and partly to the tremendous growth of bacteria which took place within 14 days, especially in the limed green-manure plots. Without limestone, nitrification was delayed, nitrates not accumulating in the rye or vetch plots until the forty-second and sixty-third day samplings.

The results of the second applications of rye and vetch on the Leonardtown plots gave similar though more striking results. (Fig. 6.) The nitrate content of all the treated plots was between 190 and 245 parts per million when the green manure was turned under. Within 7 days it had decreased markedly, only to rise again, at 14 days. After another slight decrease, nitrates rose rapidly at the last two determinations and in most cases attained the same height as at the beginning of this experiment.

The effect of rye and vetch green manures on the nitrates in the field soil is shown in Figure 7. Rye green manure failed in this instance to increase the nitric nitrogen at any time during the experiment. The vetch treated plot, on the other hand, showed an increase beginning at 21 days and amounting to over 50 parts per million in 56 days.

The rapid accumulation of nitrates under optimum conditions after green manuring has been observed by many investigators. Joshi (25) found that *Crotolaria*, *Sesbania*, and cowpeas nitrified rapidly under

laboratory conditions. In 1 week nitrates were 45 to 70 parts per million; in 2 weeks, 85 to 120; and in 3 weeks, 110 to 145. Similar results were obtained by Whiting and Schoonover (42) when green clover tops were added to soil in gallon jars kept in the greenhouse. These and the results obtained by others are in accord with the data presented in this paper.

Nitrification took place in the acid plots and to practically the same extent as in the neutral plots. Hall, Miller, and Gimingham (22) reported that nitrification went on slowly in the very acid plots. They considered it as being localized around particles of limestone where the reaction was not so acid. Abbott, Conner, and Smalley (1) found that nitrification progressed as rapidly when 4,000 pounds CaCO_3 were needed as when it was added.

Brown and MacIntire (9), Temple (37), White (40), Fred and Graul (20), Stephenson (36), and others have reported active nitrification in acid soils. Olson (31) states that nitrification can take place at a pH of 3.7. In the present work the pH value of the Leonardtown soil toward the end of the first vetch green-manuring experiment was 4.2. The nitrate content at that time was only slightly lower than where limestone had also been added, showing that nitrification had gone on at this low pH value.

As a rule, nitrates were low when the bacteria were high. There are exceptions, notably in the first experiments. No explanation has been found for this with the data at hand.

The decrease in nitrates immediately after the second application of the green manures is very striking. After nine weeks they had accumulated again to the former high levels, but no higher. The bacteria at this time were low. Additional analyses for bacterial numbers and nitrates were made a few months after this experiment. The bacteria had increased somewhat and the nitrate had decreased. It is still a problem as to what became of the nitrogen added in the second green manure. At least it did not increase the amount of nitrates already in the soil.

EFFECT OF TREATMENTS ON SOIL ACIDITY

The hydrogen-ion concentration of each sample was determined to see if any change in the reaction of the soil took place during the process of decomposition. The quinhydrone electrode was used and the procedure was as follows: 5 to 10 gm. from each sample were placed in a test tube and a small quantity of quinhydrone added. A sufficient amount of distilled water was added to make the proportion of soil to water 1 : 1 to 1 : 2. A standard solution consisting of 0.09 N KCl in 0.01 N HCl and having a pH of 2.04 at 18° C. was poured into another tube. The standard solution was then connected with the soil suspension by a saturated KCl agar bridge. Clean platinum electrodes were used. The difference in electromotive force was determined on a potentiometer and correction made for temperature.

The results when rye was used as the green manure are given in Table 10.

TABLE 10.--*pH values of samples from two acid soils, limed and unlimed, in the rye green-manure series*

Days after green manuring	Collington fine sandy loam				Leonardtown clay loam			
	Control	Limed control	Rye	Limed rye	Control	Limed control	Rye	Limed rye
0	5.1	6.6	5.2	6.5	4.4	6.5	4.6	7.3
2	5.2	7.0	5.2	6.9	4.4	7.1	4.6	7.5
4	5.3	6.3	5.3	6.9	4.6	6.8	4.6	7.1
7	4.7	6.6	5.0	6.8	4.6	7.2	4.4	7.4
14	4.8	6.4	4.8	6.5	4.5	6.8	4.4	7.1
21	4.7	6.7	4.6	6.6	4.4	6.7	4.2	7.3
35	5.1	6.5	4.8	6.6	4.5	7.0	4.2	7.2
56	5.3	6.9	4.8	6.8	5.1	7.0	4.4	7.0
84	4.7	6.7	4.5	6.8	4.2	6.9	4.0	7.2
119	4.8	6.8	4.7	6.6	4.2	6.7	4.1	7.2

A definite change in the hydrogen-ion concentration of the soil took place during the experiment. The unlimed rye green-manure plots of both soils became more acid toward the end. This was probably due to the production of nitric nitrogen which, as was shown above, took place in these acid soils. Where limestone was added, the acidity, of course, was taken care of by the excess of limestone added. In comparing the limed control with the limed rye plot of the Leonardtown soil, it is seen that the pH value of the latter is higher throughout the experiment. Six months later the pH value was the same in both, being slightly above 7. As the same amount of limestone was added to both plots, the only explanation seems to be that the limestone was not evenly distributed in this particular case. The determinations as a whole leave much to be desired in the way of uniformity.

The effects produced on the pH values by vetch green manure were similar to those produced by the rye. (Table 11.) Although the experiment lasted only 56 days, there was a definite production of acidity by that time where the limestone was not added. However, in the early stages of decomposition in the Collington soil there seemed to be less acidity; that is, a production of alkali. More detailed experiments along this line are being carried on.

TABLE 11.--*pH values of samples from two acid soils, limed and unlimed, in the vetch green-manure series*

Days after green manuring	Collington fine sandy loam				Leonardtown clay loam			
	Control	Limed control	Vetch	Limed vetch	Control	Limed control	Vetch	Limed vetch
0	4.9	6.5	5.0	6.4	4.6	7.4	4.6	7.3
2	4.8	6.4	5.4	6.5	4.6	7.0	4.7	6.6
4	5.0	7.0	5.4	7.1	4.5	7.4	4.6	7.3
7	5.0	6.7	5.2	7.2	4.6	7.3	4.7	7.2
14	4.8	6.9	4.9	7.0	4.5	7.2	4.5	7.3
21	4.9	6.8	4.9	6.6	4.4	7.3	4.5	7.2
35	4.8	6.8	4.6	6.8	4.3	7.3	4.2	7.1
56	4.7	6.7	4.4	6.6	4.3	7.0	4.2	7.2

The second application of rye and vetch green manures made some six months after the first application gave similar results. Again it was noted that when vetch green manure without limestone was used less acidity was present during the first seven days, especially in the

Collington soil. The Leonardtown soil, being heavier, contains more buffer, so that the small amounts of acid and alkali produced failed to show in the determinations as made. The table giving these data on the second application of the green manures has been omitted, since it contains only confirmatory information.

The effect of green manuring upon Keysport clay loam under field conditions is shown in Table 12. If the rye and vetch green-manured plots are compared with the control for each sampling, no change in pH values is observed, except in one case. At seven days, vetch seemed to have produced some alkali; but since all the determinations show considerable fluctuations from one sampling to another, it is unsafe to place any dependence upon this determination. The Keysport soil is heavily buffered and would be expected to absorb the small amounts of acid and alkali which might be produced in the decomposition of the green manure and the mineralization of its nitrogen.

TABLE 12.—*pH values of Keysport clay loam as affected by green manuring in the field experiment*

Days after green manuring	Rye	Control	Vetch	Days after green manuring	Rye	Control	Vetch
0.....	5.5	5.5	5.6	14.....	5.8	5.8	5.9
2.....	5.0	5.1	5.4	21.....	5.8	5.7	5.8
4.....	5.6	5.5	5.7	35.....	5.6	5.6	5.6
7.....	5.6	5.6	6.1	50.....	5.3	5.5	5.5

Nitrification renders the medium wherein it takes place more acid. In these experiments the tendency was noted for the control and unlimed plots to become slightly more acid with the increase in the amount of nitrates. White (41) stated that the increase in soil acidity in his experiments could not be explained as due to nitrification. While nitrification was a factor, it was by no means the chief one. Howard (24) found that rye green manure produced an acid reaction equivalent to 300 to 450 pounds CaO per acre and vetch only half this amount.

Even in a soil containing more than 3 per cent CaCO_3 , Sackett and his associates (34) were able to detect an increase in the hydrogen-ion concentration due to the application of green manure. On the other hand, Ames and Schollenberger (2) found that both dry and green materials reduced the lime requirements at the end of three months. Other work might be referred to in which it is stated that green manure produces a more acid reaction or tends to neutralize an acid soil. The data are therefore rather conflicting. This is no doubt due to the varying conditions of the experiments. In the present work an increase in acidity is correlated with an increase in nitrate content. What effect the absorption of these nitrates by growing plants would have upon the soil acidity can not be stated. However, there is no reason to suppose that this acidity would be permanent.

SUMMARY

The effect of the addition of rye or vetch green manures to Collington fine sandy loam and to Leonardtown clay loam under optimum conditions of temperature and moisture and with and without limestone may be summarized as follows:

Green manure on both the acid and neutral plots increased the number of soil microorganisms as determined on soil-extract agar.

The greatest number usually occurred in four days and was followed by a rapid decline coincident with the disappearance of the leafy portion of the green manure.

In the acid green-manured soil the total plate counts remained low after the decline. In the soil neutralized with limestone and treated with green manure a secondary increase took place and the number remained high.

Limestone caused an increase in the total plate count five weeks after application.

A second application of green manures to the same soil six months after the first showed the same great increase in microorganisms during the first few days. This was followed by a reduction in numbers which continued to the end of the period of observation. No secondary rise took place as was noted in the first experiments.

The increase in the soil microorganisms was due to the growth of bacteria. Both the Gram-negative and Gram-positive bacteria increased greatly during the active decomposition of the green manure.

The secondary rise in bacterial numbers due to the effect of limestone was caused by an increase in the Gram-negative organisms.

The number of fungi in the acid or neutralized soils was not influenced to any great extent by the addition of green manures.

The number of actinomycetes was lower during the greatest development of the bacteria. This was due to overcrowding of the plates by certain rapidly growing bacteria. Otherwise no effect of the green manures was apparent.

Green manures stimulated the accumulation of nitrates in both the acid and neutralized soils, the amount of nitrates being slightly lower in the acid soil. Although there were many irregularities, nitrates tended to be high when the bacteria were low, and vice versa.

There was a tendency for the control soils to become slightly more acid as the nitrates accumulated. With the addition of green manure and a greater accumulation of nitrates, there was a definite increase in acidity on the sandy soil where no limestone was added. This was not so noticeable in the clay soil.

In the early stages of decomposition small but significant amounts of alkali were produced in the sandy soil which reduced the acidity. Practically no effect was observed on the clay soil.

Vetch green manure under field conditions decomposed more slowly than under greenhouse conditions, but the effects were similar to those obtained in the greenhouse. Rye green manure under these conditions produced practically no change in the number of soil microorganisms, in the amount of nitrate, or in the hydrogen-ion concentration of the soil.

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NEODIPLOGASTER PINICOLA, N. SP., A NEMA ASSOCIATED WITH THE WHITE-PINE WEEVIL IN TERMINAL SHOOTS OF THE WHITE PINE¹

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INTRODUCTION

Fuchs (4)² in 1915 first called attention to the fact that the moist frass of the mines produced by *Ips typographus* and *Hylobius abietis* is inhabited by a specific nemie fauna, the members of which are in more or less close symbiotic relationship with the beetles. He observed that some of the nemas were carried about by the beetles, either under the wing covers or in some other way. In some instances the relationship between nemas and beetles seemed to be more or less that of parasite and host. Fuchs also mentioned the fact that different genera and species of beetles have different nemas as their associates. Similar observations were published more recently by Jazentkovsky (5).

Previous to the present paper, so far as the writer is aware, no case of the association of a nema with the white-pine beetle (*Pissodes strobi*) has been recorded.

In June, 1928, R. L. Taylor, of the Bussey Institution for Research in Applied Biology, Harvard University, sent the writer terminal shoots of the white pine (*Pinus strobus*) affected by *Pissodes strobi* and harboring numerous nemas in the mines. Doctor Taylor at that time suggested that probably the same nemas also occur under the wing covers of the beetles. Further material for study was then received from Maine through the courtesy of E. G. Arzberger. As far as the writer could see, the mines of *P. strobi* harbor only one species of nema, which, however, is new and belongs to the genus *Neodiplogaster* Cobb (2).

RELATIONSHIPS OF GENUS NEODIPLOGASTER

Neodiplogaster is closely related to the genus *Diplogasteroides* of De Man (6) and also to the genus *Rhabditolaimus* established by Fuchs (4) for forms that he found in the mines of the bark beetles of Europe. Only one species of *Neodiplogaster* is known at present—*N. tropica* which Cobb (2) found in coco pods from Guatemala. The status of the genus at that time was still somewhat doubted by its author. After making a study of the present form, the writer considers *Neodiplogaster* a good genus. Its relationship to the other genera of the *Diplogasteridae* is best shown by a summary of the diagnostic characters of the various genera:

DIPLOGASTEROIDES (De Man, 6).—*Diplogasteridae* with a cylindrical undivided pharynx, at the base of which is a small dorsal tooth; male tail with numerous papillae but no bursal structures.

¹ Received for publication Mar. 18, 1930; issued July, 1930.

² Reference is made by number (italic) to Literature Cited, p. 129.

RHABDITOLAIMUS (Fuchs, 4).—Diplogasteridae similar to *Diplogasteroides*, but with the pharynx unarmed; male tail with small bursal membranes.

NEODIPLOGASTER (Cobb, 2).—Diplogasteridae with the pharynx divided into a short but wide anterior section and a longer cylindrical posterior section; on the bottom of the anterior part a single well-developed dorsal tooth; on the bottom of the posterior part two sub-dorsal cuticularized oval pieces. Male tail with numerous papillae partly connected by more or less developed bursal structures.

DEMANIELLA (Steiner, 10).—Diplogasteridae with cylindrical undivided pharynx, at the bottom of which is a large dorsal tooth; anterior esophageal bulb elongated; oral opening with conical elevation and surrounded by a number of semicircular cuticular thickenings.

ODONTOPHARYNX (De Man, 7).—Diplogasteridae with irregularly shaped, wide, deep, armed pharynx; anterior part of esophagus without distinct bulb but its lumen throughout its entire length with highly cuticularized walls, and the esophageal tissue with strong radial muscles. Male tail with numerous papillae but no bursal structures.

CLASSIFICATION OF THE DIPLOGASTERIDAE

As against Baylis and Daubney (1, p. 41-42, 109-110), the writer holds the opinion that *Diplogaster*, *Rhabditolaimus*, *Odontopharynx*, *Demaniella*, *Neodiplogaster*, and *Diplogasteroides* are good genera, each including perhaps a number of species. They form a natural family (the *Diplogasteridae*), in which the structure of the posterior part of the esophagus is the chief distinguishing characteristic. *Rhabditolaimus* and *Diplogasteroides*, which are among those genera of the *Diplogasteridae* that show a definite relationship to the genus *Rhabditis* (family *Rhabditidae*), have the typical diplogastroid esophagus—that is to say, the terminal bulb is without radial muscle fibers and has no valvular apparatus.

The subgenus *Rhabditella*, of the genus *Rhabditis*, may be considered a connecting link between *Rhabditolaimus* and *Diplogasteroides* on the one hand and *Rhabditis* on the other, for in *Rhabditella* the male tail end very closely resembles that of either of the two diplogastroid genera. *Rhabditella* is nevertheless clearly distinguished from both of these *Diplogasteridae* by the shape of the posterior part of the esophagus, the terminal bulb of which has a definite valvular apparatus.

The *Diplogasteridae* possess additional distinguishing characters in the male copulatory apparatus with its spicula and gubernaculum and in the arrangement and shape of the papillae. The papillae, however, are sometimes supporting membranes of bursal character, thus clearly showing a relationship to *Rhabditella*. Moreover, in some forms (*Diplogasteroides*, *Rhabditolaimus*, etc.) the pharynx exhibits a close relationship to the typical *Rhabditis* pharynx.

The present classification of the *Diplogasteridae* is not considered as final. Cobb (3) has already called attention to the fact that the forms at present included in the genus *Diplogaster* represent various groups with distinct characters. The writer is convinced that in the near future these groups will be definitely separated into different genera.

HABITS OF THE WHITE-PINE WEEVIL

The white-pine weevil, *Pissodes strobi*, occurs wherever the white pine grows, but is economically important in only a part of the range of the white pine. Its principal damage is produced on this plant, although a few others like *Pinus rigida* (pitch pine), *Picea rubra* (red spruce), *P. abies* L. (Norway spruce), *Abies balsamea* L. (balsam fir), and *Tsuga canadensis* L. (hemlock) may also be attacked. The beetle causes in the pine the abnormal development that is called "cabbage" or "pasture" pine. It attacks the terminal shoots of the host plants, killing them off; new leading shoots may then be developed and in turn killed, the result being that often a large number of new terminal shoots are formed which give the host plant the forked and crooked aspect previously mentioned.

The white-pine weevil lays its eggs in small holes that it makes in the topmost shoot of the previous year's growth. A number of eggs are usually deposited in one hole, and the newly hatched larvae gnaw small mines in the tissue below the bark. Usually only some of the eggs develop, and the mortality of the larvae is very high, presumably from starvation or parasitism. The mature larvae bore into the wood parenchyma and pupate. The adults emerge in the latter part of August or still later and are supposed to hibernate in the ground (Peirson, 9).

NEODIPLOGASTER PINICOLA N. SP.

ECONOMIC IMPORTANCE

The life cycle of the nema observed by the writer as a parasite of the white-pine weevil is not yet known, but the fact is established that the adult beetle carries specimens of the nema under its wing covers, and only by this fact can the presence of the nema in all the mines examined be understood. It is considered probable that when the beetle deposits its eggs some of the nemas slip out from under the wing covers and remain with the eggs in the new mine.

Here, perhaps, the nema comes in as a control factor of economic value by causing the high mortality in the eggs and young beetle larvae previously mentioned, although this has not yet been verified by observation or experiment. *Diplogaster* species, however, have been observed feeding on grasshopper eggs (Merrill and Ford, 8, p. 124). This fact and observations on other *Diplogasteridae* suggest the possibility that the present form may feed on the eggs of the beetles.

TECHNICAL DESCRIPTION

***Neodiplogaster pinicola*, n. sp.**

Neodiplogaster pinicola (fig. 1) is slender and of small size, not quite 1 mm. in length. The tail end of the larva and of the female is long-conical and sharply pointed. That of the male is much shorter but also conical and pointed. The cuticle is annulated, but with high magnification the annulation is resolved into series of transverse and longitudinal dots. These dots are more distinct on the lateral surface, especially at the tail end. Figure 1, F, gives a view of the arrangement of these dots. A wide undotted space always separates a double longitudinal series of dots except along the lateral line where four such series of dots are close together. (Fig. 1, F.) If, however, these series of dots are analyzed, it can be seen at once that groups of four dots alternate with groups of only two, which are placed in a transverse direction. This arrangement gives the surface a very specific character. The head end is not set off; its shape is convex-conoid. A

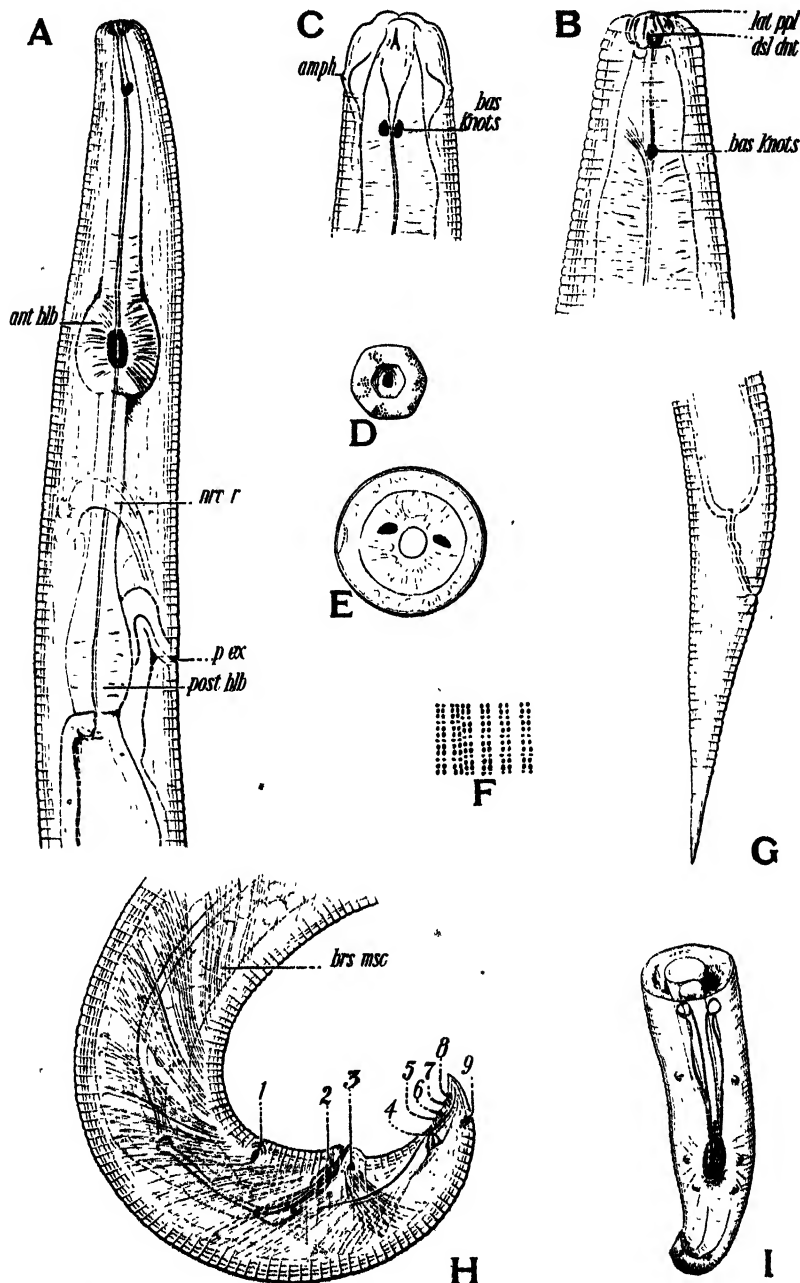


FIGURE 1.—*Neodiplogaster pinicola*, n. sp. A, Anterior end; *ant blb*, anterior esophageal bulb; *nrv r*, nervuring; *p ex*, porus excretorius; *post blb*, posterior esophageal bulb. \times about 700. B, Lateral view of head end; *bas knots*, cuticularized dorso-sublateral basal knots of pharynx; *dsi dnt*, dorsal onchium; *lat ppi*, lateral papilla. \times about 1,433. C, Medial view of head end; *bas knots*, cuticularized dorso-sublateral basal knots of pharynx; *amph*, amphid. \times about 1,433. D, Front view of head. \times about 1,433. E, Cross section in region of basal knots of pharynx. \times about 1,433. F, Arrangement of dots on cuticle. G, Tail end of female. \times about 700. H, Lateral view of male tail end; *brs msc*, bursal muscles; 1-9, male copulatory papillae. \times about 700. I, Ventral view of male tail end. \times about 700.

circle of six papillae is present. The amphids are very inconspicuous and open behind the middle of the pharynx. The latter is separated into two parts, a wide but shorter anterior one, which is striated longitudinally, and a narrow but longer posterior one. At the base of the anterior portion of the pharynx a single dorsal tooth is seen; it is quite conspicuous and curves forward. The second part of the pharynx is narrower but much longer and of somewhat cylindrical shape. Its shape in cross section was not made out. Two ellipsoidal cuticularized structures are seen at the base of this pharyngeal section, much as in *Neodiplogaster tropica*; they seem not to protrude into the pharyngeal cavity but to be located in the surrounding tissue in a somewhat dorso-sublateral position. (Fig. 1, A, B, C, E.) Their significance is not clear. The esophagus is of typical diplogasteroid shape. As can be seen in Figure 1, A, the anterior esophageal bulb is very well developed. The male sexual apparatus has a single testis, outstretched forward. The bursal muscles are strongly developed, eight pairs being located in front of the anus and two behind it. The spiculae are arcuate, exceedingly slender; the proximal ends are cephalated, the distal ends sharply pointed. The gubernaculum completely surrounds the spicula; it is of very complicated structure, as may be seen in Figure 1, H, I. A series of about nine ventro-submedial papillae was seen on each side of the male tail. The arrangement is very specific and furnishes an outstanding differentiating feature between *Neodiplogaster tropica* and the present species. The first papilla is near the middle of the spicula, the second is just in front of the anus, the third just behind it. These three papillae are not of identical size, the first one being larger than the other two. A fourth papilla is located ventro-submedially just behind the middle of the tail; it seems to support a small membranous structure, a kind of miniature bursa. A series of four much smaller papillae is seen in front of the tail end, close to the ventro-medial line. The ninth papilla, however, has a dorso-submedial position a short distance in front of the tail end. The female reproduction apparatus is amphidelph and the ovaries are reflexed. The vulva opens just behind the middle of the body.

MEASUREMENTS:

			20	22		
♀	2.6	10.1	14.4	55.5	93.8	0.907 mm. (0.834 mm.-0.980 mm.)
	1.5	2.1	2.7	3.8	2.6	
				62.5		
♂	2.8	12.	18.5	-M	92.2	0.687 mm. (0.66 mm.-0.713 mm.)
	1.5	3.4	3.5	4.7	3.4	

DIAGNOSIS OF THE NEW SPECIES.—*Neodiplogaster* differing from *Neodiplogaster tropica* mainly in the different arrangement of the male copulatory papillae, in the much more reduced bursa, and in the presence of a dorso-submedial papilla close to the end of the male tail. Wall of anterior portion of pharynx with longitudinal striae.

SUMMARY

A new nema, *Neodiplogaster pinicola*, n. sp., is described, and the affinities of this and other genera of the Diplogasteridae are reviewed. The present form was found living in the moist frass of mines produced by *Pissodes strobi*, the white-pine weevil, in terminal shoots of the white pine (*Pinus strobus*). It is suggested that this nema may be of economic importance as an enemy of its host, on the eggs and larvae of which it probably preys.

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A METHOD FOR DETERMINING THE QUANTITY OF OIL RETAINED BY CITRUS FOLIAGE AFTER SPRAYING¹

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INTRODUCTION

In order to determine the effect of oil emulsions on Satsuma orange trees and on the insects which infest their foliage, it is desirable to know how much oil remains on the foliage after spraying. Laboratory and field experiments were therefore conducted in an effort to develop a method for determining the amount of oil retained by the foliage. The method herein reported was finally adopted.

MATERIAL AND EQUIPMENT

To avoid the laborious and time-consuming operation of measuring leaves with a planimeter, the method of cutting disks from the leaves, suggested by Ginsburg,² was used. A 10 sq. cm. disk proved to be a convenient size. Including both sides of the leaf, 50 such disks make a sample having a total area of 1,000 sq. cm. Even though the determinations represent the amount of oil both on the leaf and in the leaf, it was thought advisable to base the calculations on the area sprayed.

The graduations on the Babcock bottles used were calibrated to read in cubic centimeters rather than in percentage by running a drop of mercury into the capillary, measuring its length, and then weighing it. A uniform lot was obtained that calibrated 0.002 c. c. for each division of the tube. The regular heating unit in an oven was replaced with a 100-watt electric-light bulb to make it safe for concentrating ether extracts. A standard type of centrifuge equipped with an electric heater was used.

PROCEDURE

The emulsion was applied to duplicate trees with a power sprayer. The concentration of the emulsion at the nozzle was checked by a slight modification of the Lichtenberg³ method, which has been found more satisfactory than the method in which only sulphuric acid is used. The day after application, or as soon as the spray dried, 50 leaves were clipped from various parts of each tree. A 10 sq. cm. disk was punched from each leaf. The 50 disks were placed in a 6-ounce wide-mouthed bottle, 50 c. c. of ether were added, and the

¹ Received for publication Jan. 29, 1930; issued July, 1930. This paper is based on cooperative investigation between the Agricultural Experiment Station of the Alabama Polytechnic Institute and the Alabama State Department of Agriculture.

² GINSBURG, J. M. AN APPARATUS FOR OBTAINING MEASURED AREAS OF SPRAYED FOLIAGE FOR CHEMICAL ANALYSES. *Jour. Agr. Research* 36: 1007-1009, illus. 1928.

³ LICHTENBERG, H. F. A RAPID METHOD FOR THE DETERMINATION OF FAT IN ICE CREAM. *Indus. and Engin. Chem.* 5: 786. 1913. (Letter to the editor.) This is a method for the determination of the amount of fat in ice cream. The procedure calls for 9 gm. of the sample, 20 c. c. of glacial acetic acid, and 10 c. c. of sulphuric acid. This was modified to the following: 10 c. c. of oil emulsion, 15 c. c. of glacial acetic acid, and 10 c. c. of concentrated sulphuric acid, chemically pure. The mixture is shaken after the addition of each acid. It is then centrifuged, hot water added, re-centrifuged, and read. The determination is carried out in Babcock 8 per cent milk bottles or skim-milk bottles, depending upon the concentration of the emulsion.

bottle was closed with a cork stopper and shaken by hand for one minute. The extract was filtered through a fast filter paper into a 125 c. c. Erlenmeyer flask. Another 50 c. c. portion of ether was added and the extraction repeated. The filter paper was washed with 10 c. c. of ether. The extract was concentrated to 20–25 c. c. in the open air and transferred to a Babcock skim-milk bottle. Five cubic centimeters of approximately 0.5 N sulphuric acid were added as a floating medium. (The loss of oil due to sulphonation is negligible, even if the unsulphonated residue of the oil is low.) The remainder of the ether was driven off in an electric oven at 50° C. The temperature was gradually raised to 80° to insure removal of all traces of ether. Enough hot, dilute sulphuric acid (0.5 N) was added to almost fill the bottle. It was heated to 80°–85°, centrifuged at approximately 2,000 revolutions per minute in a heated centrifuge, reheated until the oil flowed freely, and more acid was carefully added in successive portions with reheating and recentrifuging to bring the oil gradually up into the capillary tube, where it was read at room temperature with a pair of dividers.

EXPERIMENTAL RESULTS

Table 1 shows the results from samples of leaves collected from trees sprayed with an oil emulsion at a volume concentration of 3 per cent. The determinations from two trees agreed very closely, and the amount of oil extracted from the disks checked satisfactorily with the amount from the whole leaves. The determination from sample C indicates that all the oil was obtained by two successive extractions with 50 c. c. of ether.

TABLE 1.—Amount of oil extracted from disks and whole leaves collected from duplicate trees sprayed with an oil emulsion of 3 per cent concentration by volume

Sample	Method of extraction	Millionths of a cubic centimeter of oil per square centimeter on leaves from—	
		Sprayed trees	Check trees
A.....	Fifty 10 sq. cm. disks given 2 successive extractions.....	64.0	22.0
B.....	Twenty whole leaves extracted same as A.....	62.0	20.0
		65.3	20.3
		67.3	21.5
C.....	Disks from A reextracted.....	2.0	2.0
		2.0	2.0

Table 2 gives examples of the results which may be expected from the use of this method. In this table, data are shown from five trees in each of two separate blocks sprayed with different emulsions. The variations are probably due to differences in individual trees and imperfections in spraying rather than to inaccuracies in the method itself.

TABLE 2.—Amount of oil obtained from five random samples collected from two blocks of sprayed trees

Sample No	Millionths of a cubic centimeter of oil per square centimeter of leaf in --			Sample No.	Millionths of a cubic centimeter of oil per square centimeter of leaf in—		
	Block 1	Block 2	Check		Block 1	Block 2	Check
1 -----	62	60	24	4. -----	62	68	24
2 -----	62	56	24	5 -----	52	68	(*)
3 -----	58	61	21				

* Fifth sample not collected because of rain.

Allowing disks to stand in ether overnight, or extracting in a Soxhlet apparatus for six hours, did not increase the net amount of oil. These more drastic extractions merely increased the gross determination on both treated and check trees. This is undesirable in making determinations from foliage on which there is only a small amount of oil. The high melting point of the residue from check trees also makes the determination more difficult. There is no advantage in extracting with a mixture of ether and benzin in place of ether. On the contrary, it is often difficult to remove the last trace of benzin, even though it has previously been redistilled at a temperature not exceeding 60° C.

SUMMARY

A practical method is reported for determining the amount of oil retained by citrus foliage after spraying with oil emulsions. The procedure consists of the extraction of oil with ether from measured areas of foliage and its subsequent recovery and measurement in Babcock skim-milk bottles.

THE VALUE OF POTASSIUM IODIDE AS A SUPPLEMENT TO THE RATION OF GROWING CHICKS¹

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INTRODUCTION

That birds as well as mammals respond to iodine deficiencies by enlargement of the thyroid is evident from the investigations of Welch in Montana. On this subject he² says:

Goiter in poultry is very common. Flocks with a very high percentage of it have been noted in goiterous areas. The enlarged thyroid, frequently as large as the thumb, is entirely concealed by the feathers, and so far we have not observed any bad effect on the health of these fowls. The owners of goitered flocks have always reported that egg production and general health were normal. We have attempted to determine whether congenital goiter existed among newly hatched chicks but have been unable to get the material to study. No losses have been reported, at any rate, from this cause.

A detailed report of a case of simple colloid goiter in poultry has been reported by Kernkamp.³ In Minnesota, goiter in poultry, according to this report, is extremely uncommon.

In the case of obvious goiter, the medicinal use of iodine is justified and may be expected to give relief on the basis of experimental investigations on other animals. But in those localities of the country, including Illinois, in which goiter in farm animals is not endemic and is in fact of rare occurrence, the necessity or the wisdom of supplementing farm rations with some form of iodine is not self-evident and can be determined only by controlled experimental investigations. At present there is a lack of experimental evidence on the growth-promoting value of an iodine supplement to the rations of poultry. This situation prompted the experiment reported in this paper.

The experiment represents an attempt to extend the paired-feeding method to groups of three, the food intakes of each triplet being kept approximately the same. The result was not entirely successful, but nevertheless the information obtained seems to warrant publication.

EXPERIMENTAL PROCEDURE

Eighteen White Leghorn chicks, about 6½ weeks of age, were divided into six groups of three birds each in such a way that the birds in each triplet were of the same sex and of about the same weight. Five of the triplets were made up of pullets and the sixth of cockerels. At the beginning of the experiment the birds ranged in weight from 230 to 320 gm., averaging 270 gm.

All birds were fed the same basal ration⁴ of ground yellow corn 65 parts, wheat bran 10 parts, middlings 10 parts, tankage 10 parts, steamed bone meal 3 parts, sodium chloride 1 part, and charcoal 1 part.

¹ Received for publication Feb. 1, 1930; issued July, 1930.

² WELCH, H. GOITER IN FARM ANIMALS. Mont. Agr. Expt. Sta. Bul. 214, 26 p., illus. 1928.

³ KERNKAMP, H. C. H. GOITER IN POULTRY. Jour. Amer. Vet. Med. Assoc. 67:223-228, illus. 1925.

⁴ A composite sample of this ration, covering the entire period of the experiment, was analyzed with the following results: Dry substance, 92.43 per cent; crude protein, 16.31 per cent; nitrogen-free extract, 54.59 per cent; crude fiber, 3.44 per cent; ether extract, 10.99 per cent; and ash, 7.10 per cent.

TABLE 1.—*Body weights, weekly gains or losses, and food consumption of growing chicks as affected by feeding potassium iodide at two levels^a as a mineral supplement*

Item	Group 1			Group 2			Group 3		
	Check bird	Bird fed 0.5 mgm. KI	Bird fed 1 mgm. KI	Check bird	Bird fed 0.5 mgm. KI	Bird fed 1 mgm. KI	Check bird	Bird fed 0.5 mgm. KI	Bird fed 1 mgm. KI
Final weight... gm.	1,110	1,120	560	510	1,020	1,070	1,060	1,120	1,100
Initial weight... do.	320	310	310	290	280	290	270	270	270
Total gain... do.	790	810	250	220	740	780	790	850	830
Test period... days	98	98	21	21	98	98	98	98	98
Average daily gain... gm.	8.06	8.26	(11.90)	(10.48)	7.55	7.96	8.06	8.67	8.47

WEEKLY GAINS

Week	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
1	110	95	110	70	70	100	80	100	100
2	-20	65	60	70	60	80	45	40	80
3	170	60	80	80	70	80	75	60	60
4	50	100			50	70	50	30	20
5	-40	-50			-20	-30	-60	-40	-40
6	130	160			130	160	210	230	100
7	50	50			40	40	-60	-60	-80
8	50	50			-10	60	60	70	140
9	50	60			150	120	150	150	100
10	80	90			50	60	100	60	110
11	50	40			-30	40	0	60	0
12	50	30			80	120	80	70	80
13	30	40			70	-200	30	80	60
14	30	20			30	110	10	20	10
Total feed eaten...	5,335	5,335	950	1,050	6,195	6,105	5,290	6,300	6,040
Average daily ration...	54.4	54.4	45.2	50	63.2	63.2	64.2	64.3	61.6
Feed per gram gain...	6.75	6.58	(3.80)	(4.77)	8.37	7.94	7.96	7.41	7.27

Item	Group 4			Group 5			Group 6		
	Check bird	Bird fed 0.5 mgm. KI	Bird fed 1 mgm. KI	Check bird	Bird fed 0.5 mgm. KI	Bird fed 1 mgm. KI	Check bird	Bird fed 0.5 mgm. KI	Bird fed 1 mgm. KI
Final weight... gm.	920	950	1,010	930	1,020	1,040	1,240	1,120	1,140
Initial weight... do.	260	260	260	240	230	260	260	250	230
Total gain... do.	660	690	750	690	790	780	980	870	910
Test period... days	98	98	98	98	98	98	98	98	98
Average daily gain... gm.	6.73	7.04	7.65	7.04	8.06	7.96	10.00	8.88	9.28

WEEKLY GAINS

Week	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
1	70	85	65	70	60	120	140	35	70
2	50	55	70	60	70	-20	90	75	140
3	90	50	35	60	40	50	75	110	50
4	50	80	65	60	60	10	115	90	90
5	-30	-50	-5	-10	-10	-10	-80	-10	0
6	180	140	110	120	140	160	195	130	100
7	25	40	40	70	70	70	75	20	70
8	-25	-10	40	20	40	-20	10	30	100
9	130	110	50	100	60	150	120	130	50
10	60	60	50	60	80	90	70	120	90
11	-10	0	40	-10	30	30	60	30	0
12	10	-40	60	50	40	90	30	120	100
13	10	20	40	40	50	-20	60	-40	40
14	50	150	90	-10	50	80	30	30	10
Total feed eaten...	5,475	5,475	5,475	6,485	6,485	6,565	6,350	6,270	6,320
Average daily ration...	55.9	55.9	55.9	66.2	66.2	67.0	64.8	64.0	64.5
Feed per gram gain...	8.30	7.93	7.30	9.40	8.21	8.42	6.48	7.21	6.95

^a The feeding was in each case at the rate of 0.5 or 1 mgm. potassium iodide daily per 100 gm. body weight.
 This bird laid 5 eggs during the last 2 weeks of the experiment. The total weight of the eggs was 185 gm.

The birds were fed individually in cages containing approximately 4 square feet of floor space. The food intake of each of the three birds in each triplet was kept the same, or very nearly so, the amount offered being determined by the bird that ate the least. Grit and distilled water were available to all birds at all times. All birds were irradiated daily for 10 to 15 minutes with ultra-violet light from a mercury vapor quartz lamp.

In each triplet, one of the three birds received only the basal ration, a second was given, in addition to the basal ration, 0.5 mgm. of potassium iodide daily per 100 gm. of body weight, while the ration of the third bird was supplemented daily with 1 mgm. of potassium iodide per 100 gm. of body weight. The potassium iodide, in the form of an aqueous solution containing 1 mgm. of the salt per cubic centimeter, was introduced directly into the crop from a burette fitted with a rubber tube of small bore.

The birds were weighed each week, and the experiment was continued for 14 weeks. Two birds, one the control bird in Group 2, and the other the bird receiving the larger dosage of iodide in Group 1, died after three weeks from unknown causes.

EXPERIMENTAL DATA

The results of the experiment have been summarized in Table 1. Of the 5 possible comparisons of total gains between birds receiving the smaller dosage of iodide and their controls, 4 favored the iodide bird and 1 favored the control. Of the 4 possible comparisons between birds receiving the higher dosage of iodide and their controls, 3 favored the iodide bird and 1 the control. Of the 5 possible comparisons between birds receiving the two dosages of iodide, 3 favored the bird receiving the larger dosage.

With only four or five comparisons possible, any division of the results, such as was thus obtained in all cases, is sufficient to prevent the formulation of a positive conclusion as to the effect of potassium iodide on growth, since such an outcome may reasonably have occurred from chance only.

The essential negativity of the results is further revealed by making comparisons between the weekly gains of comparable birds. There are 73 possible comparisons between birds getting the lower dosage of iodide and their controls, 37.5 of which favored the iodide bird; of 62 comparisons between birds receiving the higher dosage of iodide and their controls, 33 favored the iodide birds; while of 73 comparisons between the two birds in each triplet receiving iodide, 39.5 favored the bird getting the higher dose. These results are so near the ideal outcome if chance alone operated, i. e., 36.5, 31, and 36.5, respectively, that the operation of the deliberately imposed experimental condition need not be assumed.

SUMMARY

In a feeding experiment involving 18 White Leghorn chicks, individually fed so that birds whose gains were to be compared were of approximately the same initial weight and received approximately the same amount of food, no evidence was obtained that a supplement of potassium iodide at the rate of 0.5 mgm. or of 1.0 mgm. daily per 100 gms. of body weight in any way influenced the rate of growth.

A COMPARATIVE STUDY OF THE PROTECTIVE VALUE OF CERTAIN FABRICS IN STILL AND MOVING AIR¹

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E. V. FLOYD, *Professor, Department of Physics*; and LILIAN BAKER, *Professor
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INTRODUCTION

Clothing made from wool fabrics is generally regarded as affording the body more protection than that made from cotton. This indicates that wool is a poorer conductor of heat than cotton. There are available data to prove that the wool and the cotton fibers differ greatly in their capacity to conduct heat. (See footnote 10.) Therefore, if wool fabrics are warmer than cotton there must be other influencing factors than the heat conductivity of the fibers themselves.

The heat conductivity of a fabric may differ greatly from that of the fiber from which it is made. This may be due to a number of factors, such, for example, as the construction of the fabric, the finish, and in the case of a rough surface, the position of the nap or pile in relation to the body.

The purpose of this study was to compare the relative protective ratios of various fabrics as found in still air and in wind of different velocity.

REVIEW OF LITERATURE

Many investigators have studied the protection afforded the body through clothing. Count Rumford performed experimental work of this nature in the latter part of the eighteenth century. In this work, as reported by Williams,² Rumford compared the protective value of different fibers when loosely and when tightly packed around a body. His method was to inclose the bulb of a thermometer in a known weight of fibers, raise the mass to 190° F., and note the time required for the mercury to drop a given number of degrees. This work proved that small quantities of fibers holding air in their meshes offered a greater protection from heat loss than a larger quantity of fibers packed into the same space.

LeFerve³ made a study of the protective value of clothing fabrics in currents of air at different temperatures. In this work a measured quantity of air at a known speed and temperature was driven through a calorimeter past a clothed or unclothed human body. The temperature of the air was taken before it reached the body and as it left it. LeFerve concluded that the unclothed body lost more heat in a given time than the clothed body, and that the lower the environmental temperature and the greater the speed of the wind, the greater was the loss of heat.

Bergonie and Coulier⁴ determined the time required for a vessel of warm water covered and uncovered to cool 10° C. From their

¹ Received for publication Feb. 3, 1930; issued July, 1930. Contribution No. 9 from the department of clothing and textiles, Kansas Agricultural Experiment Station.

² WILLIAMS, M. *PHILOSOPHY OF CLOTHING*. Edinburgh and Glasgow. 1890.

³ BURNS D. *AN INTRODUCTION TO BIOPHYSICS*. (With a foreword by D. Noel Paton). 435 p., illus. New York. 1921.

⁴ BURNS, D. *Op. cit.*

work they concluded that the amount of air held in a fabric determines to a great extent its protective value. Krieger, according to Pettenkofer,⁵ reported experiments conducted with single and double layers of fabrics. His method was to cover a metal vessel of hot water with a fabric and to determine the time required for the water to cool through a given number of degrees. He concluded that it was not the kind of fiber or its weight but the texture and the volume which are the principal causes of differences in heat retention by fabrics.

Rubner⁶ contributed valuable data on the heat conductivity of clothing fabrics. His work included the influence of the moisture in a fabric on its protective value. He found that moisture lowered the protective value of clothing.

Caton⁷ conducted experiments to determine the effect of weave, color, and fiber on the heat-retaining properties of fabrics. Bottles

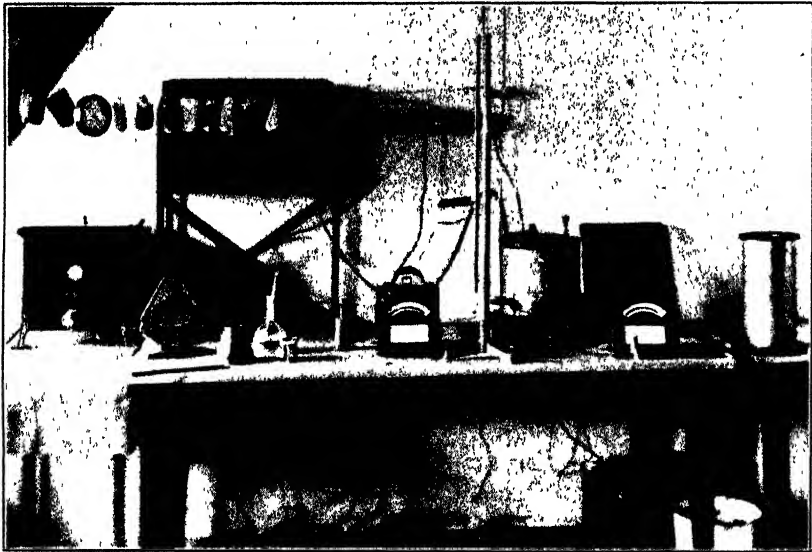


FIGURE 1.--Special apparatus used in the study of the protective value of fabrics

of warm water were covered with the fabrics under study and the time required for the bottles to cool was compared. She concluded that moisture in a fabric increased its ability to prevent loss of heat from a body.

Clark⁸ measured the protective value of certain clothing fabrics. The study included analyses of the fabrics used and determination of the electrical energy that would be necessary to keep a fabric-covered body at a temperature of 36° C. under rigidly controlled conditions of relative humidity and temperature. Thirteen fabrics were employed in this work. The conclusions drawn were that the protective ratio of a clothing fabric can be determined in terms of electrical

⁵ PETTENKOFER, MAX VON. THE RELATION OF THE AIR TO THE CLOTHES WE WEAR, THE HOUSE WE LIVE IN AND THE SOIL WE DWELL ON. [Typewritten copy on file, Kansas State Agricultural College, Manhattan.]

⁶ RUBNER, M., GRUBER M. VON, FICKER, M., editors. HANDBUCH DER HYGIENE, UNTER MITWIRKUNG VON R. ABEL, J. BOETHKE [u. a.]. v. illus. Leipzig.

⁷ CATON, F. THE HYGIENE OF WOMEN'S UNDERWEAR. (Thesis, Univ. of Missouri). 1920.

⁸ CLARK, F. R. THE PROTECTIVE VALUE OF CERTAIN CLOTHING FABRICS. [Masters thesis. Copy on file Kansas State Agricultural College, Manhattan.] 1925.

energy; that the greater the percentage of moisture in a fabric the lower is the protective value; and that there was in the fabric tested no consistent correlation between the protective ratio and the mass per unit area of the fabric.

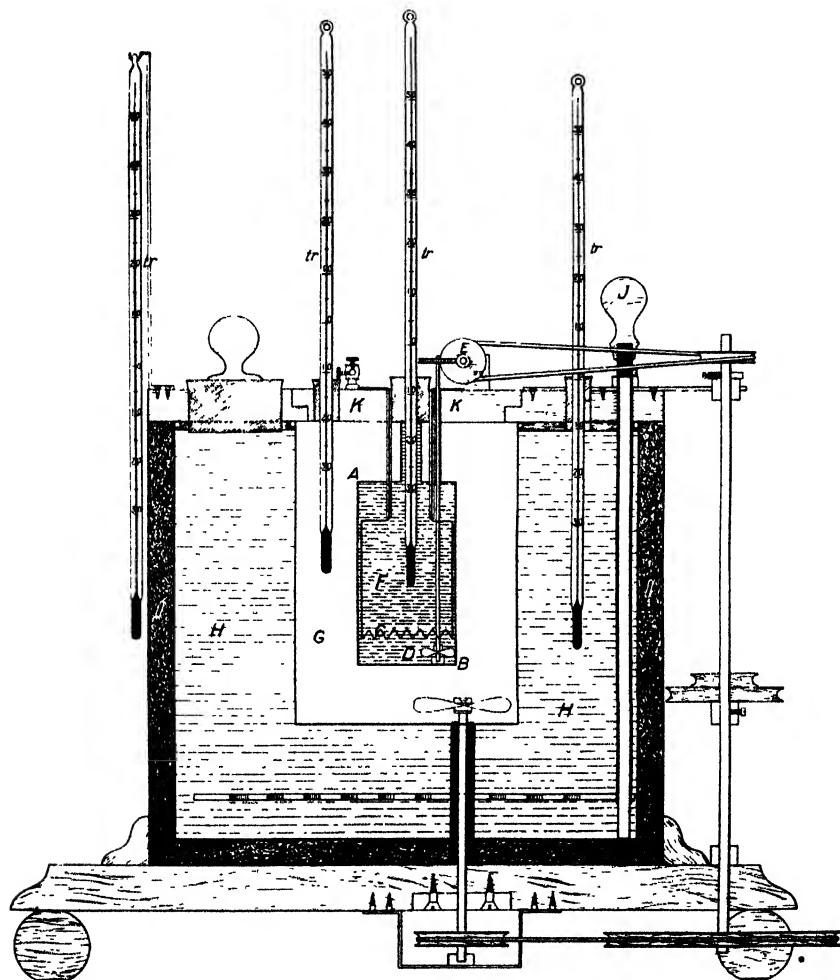


FIGURE 2.—Detailed drawing of calorimeter: A-B, Oil-filled copper cylinder 2 inches in diameter and 4 inches long, C, heating coil terminating in binding posts on calorimeter top, D, a 4-blade stirrer operated by worm gear E and the system of pulleys, shafts, and belts shown in the diagram, F, transformer oil filling the copper cylinder; G, air maintained in slow motion by the small electric fan as shown, H, water jacket surrounding the air calorimeter; J, stirrer for water in jacket; K, wooden top containing rubber gaskets which seal the air calorimeter, *tr*, thermometers for reading temperatures of room, water, air in the calorimeter, and oil

EXPERIMENTAL METHODS

The apparatus for the present study was constructed from the same design and the method of procedure was identical with that used by Clark.⁹ The fabric analyses as reported by Clark were accepted in this work. An air-tight box in which the relative humidity

⁹ CLARK, F. R. See footnote 8.

and temperature were easily controlled aided greatly in conditioning the fabrics.

The method used was that of compensating electrically the heat loss from an oil-filled copper cylinder placed in a well-insulated calorimeter. The special apparatus is shown as a whole in Figure 1 and the calorimeter in detail in Figure 2.

The experiments were performed in a room equipped in such a manner that the temperature and relative humidity of the air could be measured and regulated. A relative humidity of 40 ± 1 per cent and a temperature of $25^\circ \pm 1^\circ$ C. were used for this work. The fabrics were kept in the constant-humidity box under these conditions, and air of this relative humidity and temperature was blown into the air chamber of the calorimeter immediately before each experiment. The chamber was practically air-tight, and the temperature was held constant by means of the water jacket. The oil in the cylinder and the air surrounding it were stirred just enough to keep the temperature even throughout. The temperatures were read from thermometers accurate to 0.1° C.

A 6 to 9 volt storage battery was wired through an adjustable resistance to the heating element. By means of the adjustable resistance, an ammeter, and voltmeter the amount of electrical energy necessary to compensate for the heat losses from the oil-filled cylinder to its environment could be determined. It was found possible by this means to so accurately compensate for the heat loss that no perceptible change in temperature could be detected with the thermometer used. The electrical energy required to maintain the bare cylinder at 36° C. was recorded. The copper cylinder was then covered with the fabric under study and replaced in the calorimeter. The compensating energy was again determined for the fabric-covered cylinder.

The ratio of the compensating energy of the bare cylinder to that of the clothed cylinder was taken as the ratio of protection for the fabric.

The following fabrics commonly used in clothing were studied: Canton flannel, nap in and nap out; knit cotton underwear, knit infant's vest, wool and cotton, navy-blue flannel, and gray astrakhan, pile in and pile out.

The analyses of these fabrics included the kind of fiber, method of construction, weight per square yard, picks and ends, breaking strength, twist per inch of yarn, and the mass per unit area. These were determined for the dry fabrics in grams per square meter and in ounces per square yard.

The percentage of enmeshed air in the fabrics was determined by means of the thickness, the total volume, and the volume of the air.¹⁰ The thickness of the fabric containing the enmeshed air was found in the following manner: By means of a micrometer microscope the thickness of the fabric was measured, and the following weights were then applied over a space 1 inch square: 2.12, 4.12, 10.12, 20.12, 40.12, and 160.12 gm. A curve was plotted from these weights and measurements. This curve was extended by

¹⁰ MALONEY, M. A. THE VOLUME OF ENMESHED AIR AS A FACTOR IN THE PROTECTIVE VALUE OF CLOTHING. [Unpublished manuscript. Clothing and Textiles Dept., Kansas State Agricultural College, Manhattan.]

extrapolation to where it intercepted the zero line. The values thus determined were taken as the thickness of the fabric containing the volume of enmeshed air with no weight applied.

The amount of air in 100 sq. cm. of fabric was determined by measuring the volume of water replaced by the fabric after all the air that could be pressed out had been removed. The total volume, or volume of the fabric and air, minus the volume of the fabric, gave the volume of enmeshed air. The percentage of enmeshed air was found by dividing the volume of enmeshed air by the total volume.

TABLE 1.—Quantity of enmeshed air, thickness of fabric, mass in ounces per square yard, protective ratio, and protective values in per cent of various fabrics when tested in still air and in winds of differing velocities

Fabric	Thick- ness	Mass	En- meshed air	Speed of wind	Protec- tive ratio	Protec- tion
	Mm	Oz. per sq. yd.	Per cent	Miles per hour		Per cent
Canton flannel (nap side in)	2.22	10.00	87.9	0	1.15	13.74
White knit goods (wool and cotton)	2.1	11.31	83.77		1.18	15.47
White knit underwear (all cotton)	1.92	11.14	81.96		1.18	15.64
Blue flannel (wool)	1.03	4.71	89.93		1.20	17.06
Canton flannel (nap side out)	2.22	10.00	87.9	2.2	1.24	19.84
Gray astrakhan (pile side in)	4.15	15.70	89.25		1.33	24.86
Gray astrakhan (pile side out)	4.15	15.70	89.25		1.39	28.40
Canton flannel (nap side in)					1.16	14.52
White knit goods (wool and cotton)				2.2	1.210	17.30
White knit underwear (all cotton)					1.213	17.53
Blue flannel (wool)					1.23	18.88
Canton flannel (nap side out)					1.303	23.28
Gray astrakhan (pile side in)				2.2	1.402	28.08
Gray astrakhan (pile side out)					1.43	30.11
Canton flannel (nap side in)					1.18	15.47
White knit goods (wool and cotton)					1.28	21.80
White knit underwear (all cotton)				2.2	1.29	22.78
Blue flannel (wool)					1.31	23.07
Canton flannel (nap side out)					1.45	30.92
Gray astrakhan (pile side in)					1.49	33.04
Gray astrakhan (pile side out)				4.5	1.59	37.04
Canton flannel (nap side in)					1.25	20.49
White knit goods (wool and cotton)					1.4	28.51
White knit underwear (all cotton)					1.41	28.93
Blue flannel (wool)				4.5	1.42	30.12
Canton flannel (nap side out)					1.53	38.56
Gray astrakhan (pile side in)					1.56	36.04
Gray astrakhan (pile side out)					1.72	42.03
Canton flannel (nap side in)				8.5	1.26	21.29
White knit goods (wool and cotton)					1.42	29.81
White knit underwear (all cotton)					1.50	33.33
Blue flannel (wool)					1.49	35.45
Canton flannel (nap side out)				8.5	1.63	38.71
Gray astrakhan (pile side in)					1.81	44.69
Gray astrakhan (pile side out)					1.82	45.12

EXPERIMENTAL DATA

The work in still air indicates that the percentage of enmeshed air in a fabric determines to a great extent the protective ratio of that fabric, or the protection that a fabric will afford in preventing loss of heat from a body.

If the protective ratio of the fabric in still air is a function of the enmeshed air, it is important to know the effect of air in motion on this ratio. To determine this point an investigation was made to test certain of these same fabrics in air currents of different velocities. The oil cylinder, fabric capsules, and thermometers used in the first part of the work were employed in this study.

In order to provide air currents a wind tunnel was constructed which contained a motor-driven fan, the oil-filled copper cylinder, and the necessary thermometers, hygrometer, humidifying mechanism, and anemometer. A longitudinal section of this apparatus is shown in Figure 3.

The tunnel (fig. 3) was constructed of six joints and four elbows of 7-inch stovepipe. These were joined in such a manner as to provide an air-tight continuous path for the air current. One of the sections of pipe (A) was insulated and fitted to receive thermometers, the hygrometer, and the oil-filled copper cylinder. Another section (B) was jacketed in such a manner as to permit a circulation of water around the pipe so that the temperature of the air in the tunnel could be controlled. Air currents in the tunnel were created by means of a 4-blade fan (C) placed near one elbow and driven by an electric motor (D) located on the outside. The velocity of the moving air was deter-

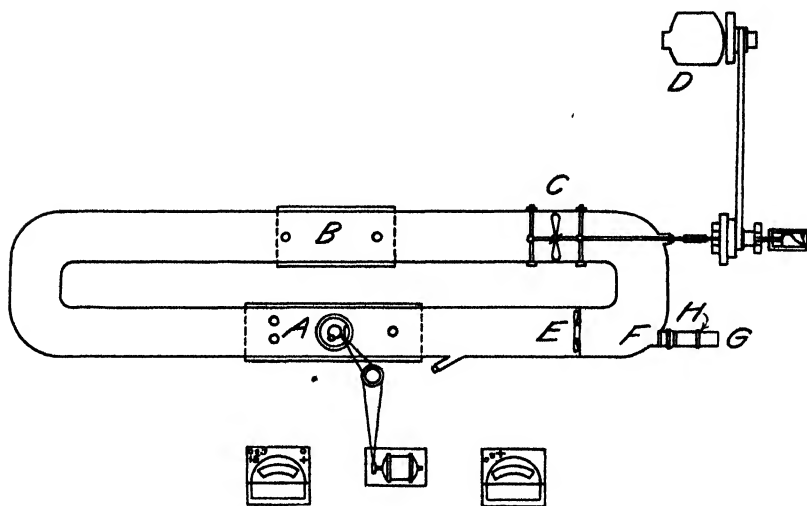


FIGURE 3.—Longitudinal section of wind tunnel. A, insulated section containing thermometers, hygrometer, and oil-filled copper cylinder; B, section of tunnel surrounded with water jacket; C, 4-blade fan; D, electric motor to drive fan; E, anemometer; F, glass window; G, flash light, H, magnifying glass; I, wet-bulb thermometer; J, oil-filled copper cylinder. Scale, three-fourths inch to 1 foot

mined by means of a 6-inch Keuffel and Esser anemometer (E) placed in the tunnel in such a manner that its readings could be taken through a glass window (F) in the wall of the tunnel. This was done with the aid of a flash light (G) and a magnifying glass (H). Figure 4 shows the apparatus as first set up. The small motor was later changed for a quarter-horse power direct-current motor. The different speeds were obtained by means of a combination of pulleys. It was possible to obtain seven different wind speeds varying from 2 to 18 miles per hour. Only four of these were used.

Experiments showed that with a given combination of pulleys the wind current varied not more than 0.6 mile per hour. The relative humidity of the air in the tunnel could be easily controlled. The temperature of the tunnel was best controlled by regulating that of the room. If the temperature of the room could not be controlled, that of the air in the tunnel was regulated with difficulty by circulating water through the jacket covering one section of the pipe.

The air in the tunnel was set in motion and regulated to 40 ± 1 per cent relative humidity at 25°C . The procedure for testing the fabrics was the same as that followed in the first part of the work.

Table 1 shows the percentage of enmeshed air, the thickness of the fabric, mass in ounces per yard, and the protective ratio and protective value in terms of percentage for the fabrics in still air and in air moving at the rate of 2.2, 3, 4.5, and 8.5 miles per hour. The variation in velocity of the wind due to the variation in electrical current made it somewhat difficult to secure closely agreeing checks and resulted in slight irregularities in the data. The fabrics are listed in the order of their protective ratio. It will be noticed that this order is the same both in still air and in air moving at the speeds tested. This does not mean that the electrical energy required to compensate for heat loss decreases as the wind speed increases, but that the electrical energy required to compensate for the heat loss of the unclothed body

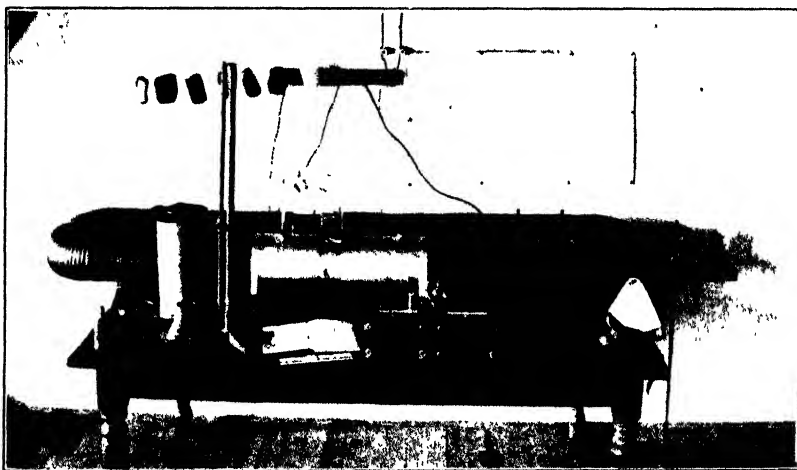


FIGURE 4 Wind tunnel and auxiliary apparatus

increases more rapidly as the wind speed increases than does that for the clothed body, resulting in a greater difference and a higher protective ratio.

In every case the Canton flannel (all cotton) and gray astrakhan (knit cotton back and wool pile) increased in protective ratio when the nap or pile was exposed to the wind or still air and the plain surface was next to the body.

Placing the pile or nap on the inside lowered the insulation possibly by (1) exposing the larger mass of the fiber of the fabric to the lower temperature, and (2) crushing the pile or nap by contact with the body and thus reducing the amount of enmeshed air in the fabric.

Table 2 shows the rating of the fabrics on the basis of their protective ratio, percentage of enmeshed air, thickness, and mass. It will be noticed that wool flannel ranks lowest in mass and thickness, fifth in percentage of enmeshed air, and fourth in protective ratio. Canton flannel is second in mass, fourth in thickness, and third in percentage of enmeshed air. This fabric ranks lowest in protective ratio when the nap is next to the body and fifth when the nap is out.

It would be necessary to handle more fabrics and to compare certain wool fabrics with other wool fabrics and certain cotton fabrics with other cotton fabrics in order to determine the relationship of the percentage of enmeshed air to the protective ratio for a group.

TABLE 2.—Numerical ratings of protective value, percentage of enmeshed air, thickness, and mass of various fabrics listed in order of protective value, beginning with the least protective

Fabric	Protective ratio		En-meshed air	Thick-ness	Mass
	Still air	Moving air			
			Per cent	Mm.	Oz. per sq yd
Canton flannel (all cotton; nap turned in) . . .	1	1	3	4	2
White knit goods (wool and cotton)	2	2	2	3	3
White knit (all cotton)	3	3	1	2	4
Wool flannel (all wool)	4	4	5	1	1
Canton flannel (nap turned out)	5	5	3	4	2
Gray astrakhan (knit cotton back, wool pile, pile turned in)	6	6	4	5	5
Gray astrakhan (pile turned out)	7	7	4	5	5

Interesting problems suggested by the study are: The determination of the heat conductivity of certain types of clothing fabrics; the effect of fitting, the effect of layers, and the effect of relative humidity and temperature on the protective value of clothing fabrics.

CONCLUSIONS

The following conclusions are drawn from the data collected under the conditions of this study:

The rating of the fabrics on the basis of increasing protective ratio was found to be the same in still air and in air moving at 2.2, 3, 4.5, and 8.5 miles an hour.

The protective ratio of a fabric increases as the speed of the wind increases. An increase from 0 to 8.5 miles an hour corresponds to an average increase in protection of 5.67 per cent.

When made into closely fitting covers, fabrics having a pile or nap afforded greater protection when the smooth surface was next to the body. This was found to be true in determinations made in both still and moving air.

THE LIMITS OF ERROR OF THE BABCOCK TEST FOR CREAM¹

By W. H. MARTIN, *Professor of Dairy Husbandry*, A. C. FAY, *Associate Professor of Bacteriology*, and K. M. RENNER, *Instructor of Dairy Husbandry, Kansas Agricultural Experiment Station*

INTRODUCTION

Since its invention in 1890 the Babcock test for milk and cream has superseded all other fat tests in the United States and Canada. In this country it is now recognized by the Association of Agricultural Chemists as a standard method for butterfat determination. In Australia, New Zealand, South Africa, and the Argentine the Babcock test is used almost exclusively.

Recognizing the necessity of protecting the financial interests of both sellers and buyers of butterfat, most States have passed laws which provide for the examination of cream testers to determine their proficiency. Most of these laws also stipulate the use of accurate glassware, weights, and other equipment, which must be approved. A system of periodic inspection by State authorities aims to insure efficient and honest testing.

As used to-day, the Babcock test is essentially the same as it was when introduced. The accuracy of the test has been checked from time to time by various investigators. Webster (12, p. 17)² was one of the first to determine the value of the meniscus. He concluded that readings taken at the top, middle, or bottom of the meniscus did not give true percentages of butterfat as determined by the gravimetric extraction method. It remained for Hunziker and his associates (6) in 1910 to suggest glymol as a means of eliminating the meniscus in reading cream tests. A few years later Spitzer and Epple (11), and Doan and his coworkers (2), in comparing the cream tests made by the Babcock and the fat-extraction methods, reported that readings with glymol approached closely the chemical analysis.

Another phase of cream testing which has received some attention is the effect of souring on the test of cream. Farrington (3, p. 5), in studying this problem, reported no difference in the tests from a can of cream before and after souring. Hunziker and his associates (5) also demonstrated conclusively that there was no increase in the test of cream after souring. Their work showed, however, that in some instances where cans of cream were allowed to stand uncovered for a considerable time in a warm place there was a slight increase in the cream test due to evaporation.

Comparisons have been made by Dahlberg and his coworkers (1, p. 29), in which the Babcock test was checked against the Roese-Gottlieb and the Gerber methods. This work showed that duplicate Roese-Gottlieb tests made in the same laboratory generally agreed

¹ Received for publication Mar. 5, 1930; issued July, 1930. Contribution No. 68 from the department of dairy husbandry and No. 119 from the department of bacteriology, Kansas Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 159.

within 0.16 per cent and tests made in different laboratories did not vary more than 0.5 per cent. The average of all the tests made by the Babcock and the Gerber methods was accurate to within 0.4 per cent or less. Ross and McInerney (9) found in testing 64 samples of cream by the Babcock and ether-extraction methods that 35 tests checked within 0.29 and only 8 varied more than 0.5 per cent. A conclusion was reached by Siegmund and Craig (10) that Babcock tests for cream gave readings slightly higher than the ether-extraction method due to the inclusion of some water and acid in the fat column. Nelson (8), in comparing 2,000 Babcock tests on market milk, found that the probable error of ± 0.02 per cent was due mainly to the method of reading.

PURPOSE OF THIS INVESTIGATION

Considering the stipulations of the various State laws relative to the limits of error of fat tests, it seemed advisable to ascertain the normal fluctuation to be expected in applying the Babcock method. Obviously, it would be unfair to require an apprentice tester to attain a degree of accuracy that can not be attained by an experienced technician. On the other hand, if the limits of error permitted by any State law are too great, the intent of the law is defeated by allowing careless and incompetent testers in the field.

The purpose of this investigation, then, is to measure the degree of normal fluctuation that may be expected in fat testing, with the ultimate aim of creating a better basis of judging the permissible limits of error.

In this discussion the term "error" means any deviation from the true fat content as far as it is obtainable by the Babcock method. For a given can of cream there is only one true value for the fat content, and any other value is in error whether the deviation be due to careless technic or to factors beyond the control of the operator. It is reasonable to expect that several samples taken from the same can of cream may contain slightly different percentages of fat. The degree of this error, of course, will be largely dependent on the thoroughness with which the cream has been agitated, but in any case some variation may be expected. These variations may result from the cream being too sour, thus rendering it difficult to procure a fair sample. Other errors undoubtedly result from imperfect weighing of the 9 gm. sample and from reading imperfect tests in which the fat column has been too badly charred by the acid. Individuals may read the same test differently. The extent of this divergence depends somewhat on the degree of experience of those reading the tests, but varies also among those who have had extensive experience.

This experiment has been organized so as to segregate some of these sources of error in order to measure their relative magnitude.

PLAN OF THE EXPERIMENT

The experiments reported in this paper have been so arranged as to measure the expected limits of error of the fat tests, the variation in readings by several persons, the error which results from careless or hasty reading, and the effect of souring on the test.

A large number of Babcock determinations were made by an experienced tester on each of two 10-gallon cans of cream, and the tests were read by five persons. From the data collected it was possible to determine the extent and degree of variability of this test in the hands of a skilled operator. Mojonnier (7, *p. 11*) tests on these samples of cream also permitted a comparison of the Babcock and ether-extraction methods. Two other groups of identical samples were submitted to three laboratories in such a way as to eliminate the psychological factor of knowing the identity of the samples. Statistical analysis of the data not only reveals the extent and degree of variation as affected by the various segregated factors, but gives some basis of judging the limits within which normal variation may be expected.

In taking all the samples for the experiments reported in this paper, unusual precautions were used in order to render the replicate samples as nearly identical as possible. The cream to be sampled was first poured back and forth from one can to another 10 times, and kept constantly in motion with a stirring rod while 8 to 10 pint samples were removed with a dipper. The cream was again poured back and forth 10 times before another group of 8 to 10 samples was removed. This process was repeated until the required number of samples was obtained.

The Babcock test was made according to the method of the American Dairy Science Association (4). All test bottles used were calibrated. The Mojonnier (7) tests were run according to the directions which are supplied with the machine.

RESULTS

VARIATION IN THE READINGS OF DIFFERENT PERSONS

SWEET CREAM

Eight 1-pint samples of sweet cream were taken according to the method previously described, and 12 replicate Babcock tests were made on each sample. Each test bottle was passed down a line of five readers, each of whom read and recorded his test privately without knowledge of the value given by other readers. The readings were made in such a manner that not more than one minute elapsed between the first and last readings on any test bottle. There were 96 tests and 456 readings made on this can of sweet cream. (One reader failed to read the tests on two of the samples.)

Some variation may be expected in the results of this method even when in the hands of a skilled operator or an experienced reader. The values established by the mean test plus or minus 3.2 times the probable error were arbitrarily accepted as marking the upper and lower limits between which a tester or reader is practically certain (30 to 1 chance) that any average of duplicate determinations will fall. That is to say, the operator can be reasonably sure that in any reading outside of these limits the deviation is due to some factor other than chance variation. In the subsequent discussion of the data the limits established by a 30 to 1 chance will be regarded as the limits of practical certainty.

Table 1 shows the minimum, maximum, and mean readings reported on each of eight samples as read by five readers. For example, of

the 12 tests on sample 1 read by five people, the lowest test reported by anyone was 41.50, the highest 42.50, and the average of the 60 readings 41.97 per cent. Similarly, Table 1 shows the minimum, maximum, and mean of the 456 readings on all samples to be 41, 42.50, and 41.76 per cent respectively.

It must be borne in mind that the extreme readings are based on single tests and are not the averages of duplicate determinations. Since the average of the 456 readings of 96 tests, 41.76 per cent, is as near to the true test on this can of cream as these data will afford, it may be seen that the normal variation of single tests may account for readings as low as 41 or as high as 42.50 per cent fat.

The individual readings of each person were treated statistically and the values for 3.2 times the probable error of duplicate tests included in Table 1. An examination of these figures shows that most of them range between ± 0.45 per cent, and that the value for all readers on all samples was ± 0.444 per cent. That is to say, it is practically certain that the average of duplicate readings would be between the limits of 41.76 ± 0.444 per cent fat.

The results reported by readers 1 and 2, who were more experienced with the test, showed less variation than those reported by the other readers. However, a low degree of variation is not necessarily an indication of more accurate readings. Undoubtedly there was some variation in the actual fat contained in the necks of the test bottles. The reporting of exactly the same value for each test bottle would indicate no variation, but might still involve erroneous reading. Nevertheless, it is logical to assume that the readings reported by the most skilled technicians (1 and 2) more nearly approximate the actual variation of the tests themselves, and that the higher variation reported by the other readers was due to less precision in reading. Readers 1 and 2 were practically certain not to make errors on duplicate readings in excess of ± 0.385 and ± 0.390 per cent from the average test. For readers 3, 4, and 5 any average of duplicate tests within ± 0.434 , ± 0.483 , and ± 0.455 per cent fat from the mean test might be due to normal variation. Incidentally, it is of interest to note that the limits of variability in reading the tests were correlated with the extent of experience of the readers.

The average of 30 replicate Mojonnier (7) tests on this can of cream was 41.8726, a value which exceeds the average of all Babcock readings by 0.1126 per cent fat.

TABLE 1.—Limits of normal variation of 456 readings of 96 fat tests on a single can of cream

Sample No.	Fat readings, all readers			Limits of a practical certainty \pm (30 to 1 chance) in reading duplicate fat tests* by—					
	Minimum	Maximum	Mean	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	All readers
	Per cent	Per cent	Per cent						
1.....	41.50	42.50	41.97	0.220	0.233	0.233	0.371	0.425	0.363
2.....	41.00	42.50	41.77	.435	.348	.380	.473	.288	.450
3.....	41.00	42.25	41.71	.240	.326	.278	.419	.480	.409
4.....	41.00	42.25	41.70	.249	.381	.390	.360	.700	.430
5.....	41.25	42.50	41.79	.422	.390	.348	.452		.420
6.....	41.50	42.25	41.83	.348	.288	.313	.300		.330
7.....	41.25	42.25	41.73	.264	.336	.361	.374	.259	.330
8.....	41.25	42.00	41.60	.358	.102	.240	.329	.188	.298
All samples.....	41.00	42.50	41.76	.385	.390	.434	.453	.455	.444

* $3.2 \times$ probable error of duplicate tests.

SWEET AND SOUR CREAM

The results obtained from the work reported in Table 1 indicated that a repetition of the experiment with some changes would be advisable. In Table 2 are reported the results of an experiment similar to the one reported in Table 1, except that ten pint samples were taken from a can of sweet cream and 16 Babcock tests were made from each sample. The can of cream was then placed at room temperature until it soured to a thick curdy consistency with an acidity of 0.45 per cent calculated as lactic acid, after which 10 more pint samples were taken and 16 tests made on each sample. Five persons read each test as in the first experiment. There were, therefore, 320 tests on a single can of cream, each read by 5 persons; half of the tests were on sweet cream and half on the same cream after it had soured. The results in Table 2 are based on the 1,599 readings (one broken) from this can of cream.

TABLE 2.--Limits of variation of 1,599 readings of 320 tests on cream before and after souring

Sample	Sample No.	Fat readings, all readers			Limits of a practical certainty \pm (30 to 1 chance) in reading duplicate tests* by—					
		Minimum	Maximum	Mean	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	All readers
		Per cent	Per cent	Per cent						
Sweet cream	1	36.00	37.50	36.98	0.364	0.230	0.313	0.454	0.643	0.442
	2	36.50	37.50	37.08	.323	.352	.326	.188	.336	.350
	3	36.75	37.50	37.14	.259	.313	.297	.201	.326	.323
	4	36.25	37.50	37.10	.336	.211	.380	.297	.473	.382
	5	36.50	37.50	37.03	.339	.198	.182	.380	.553	.390
	6	36.00	37.50	36.87	.352	.374	.553	.313	.534	.479
	7	36.50	38.00	37.14	.339	.320	.534	.332	.553	.437
	8	36.25	37.50	37.05	.307	.000	.224	.172	.470	.311
	9	36.00	37.50	36.99	.371	.345	.390	.358	.470	.421
	10	36.25	37.50	37.02	.368	.179	.294	.276	.384	.384
Same cream after souring	11	36.00	37.75	37.03	.518	.348	.528	.508	.489	.541
	12	36.00	37.50	36.94	.339	.265	.310	.294	.457	.403
	13	36.25	37.50	36.84	.339	.460	.380	.371	.396	.435
	14	36.00	37.50	36.85	.361	.246	.403	.083	.524	.490
	15	36.25	37.25	36.83	.160	.207	.348	.368	.425	.370
	16	36.00	37.50	36.89	.428	.320	.403	.438	.409	.445
	17	36.50	37.50	36.97	.361	.371	.371	.339	.377	.381
	18	36.00	37.50	36.84	.457	.419	.409	.348	.582	.454
	19	36.25	37.50	36.91	.457	.422	.400	.323	.489	.447
	20	36.50	37.50	36.92	.227	.208	.358	.204	.310	.330
All samples sweet cream		36.00	38.00	37.04	.356	.309	.415	.338	.536	.413
All samples sour cream		36.00	37.75	36.89	.399	.382	.417	.392	.491	.443

* $3.2 \times$ probable error of duplicate tests

Most of the readings on these tests ranged between 36 and 37.50 per cent, a divergence which is comparable to the results reported in Table 1. There were two readings (not tests) reported by one person of 37.75 and 38 per cent, but his readings were not substantiated by the other readers of the same test bottle.

The values for 3.2 times the probable error of duplicate tests confirm the values reported in Table 1. A comparison of the values for sweet cream in Table 2 with those in Table 1 indicates that each reader has reduced slightly the limits of normal variation of his readings. This may be partly due to the experience obtained in the preceding experiment and partly to the fact that the cream had a slightly lower test.

The two parts of the experiment with sweet and sour cream were performed under as nearly identical conditions as possible. The same technician (No. 2) performed the tests, using the same methods and equipment. When the results for sweet and sour cream in Table 2 are compared, it is at once evident that the sweet cream was given a higher test than the same cream after souring. The Mojonnier tests before and after the cream had soured are also noticeably different, being 37.6170 and 37.1125, respectively. The grand averages of the Babcock tests reported by reader No. 2 were 37.050 ± 0.1365 and 36.993 ± 0.1691 per cent fat for the sweet and sour cream, respectively. The probable error values were based on 160 tests in each case.

The question immediately arises whether the difference of 0.057 is sufficiently large to justify the conclusion that the sour cream contained less fat than the sweet. On calculating, it is found that the probable error of the difference is ± 0.2173 . Since the probable error of the difference between the mean tests for sweet and sour cream (0.2173) is nearly four times the actual difference, it is at once evident that the disparity between the means is well within the limits of normal variation.

If the probable error values in Table 2 are used as an index to the relative degree of variability of the tests on sweet and sour cream, it is noted that the values of all but one reader (No. 6) were higher on the sour cream. The greater difficulty of procuring a fair sample on thick curdled cream is no doubt responsible for the slightly greater variation evidenced in these results.

VARIATIONS OF THE TEST IN THE HANDS OF AN EXPERIENCED TECHNICIAN

Reader No. 2 made and read the tests in both experiments (Tables 1 and 2), so that his results are wholly applicable for interpreting the error of the method, whereas the results of the other readers are valuable only as a measure of the error of reading. The probable error of single tests based on his readings in the first experiment was ± 0.172 per cent fat. In reading the 320 tests on sweet and sour cream, normal variation accounted for probable error values of ± 0.136 and ± 0.169 , respectively. By calculating the values for 3.2 times the probable error of duplicate determinations, it is found that there is a 30 to 1 chance that the normal variation of his 96 tests reported in Table 1 would not introduce an error of more than 0.390 per cent fat. In other words, he could be practically certain that the average of 2 tests would be within 0.390 of the average of 96 tests. Similarly, from his results on the sweet and the sour cream (Table 2), he could be practically certain that the average of duplicate tests would not be in error more than 0.309 or 0.382 from the result obtained by averaging 160 tests on each.

The application of the probable error of one technician's work to that of another, of course, must be done with reservations. It is evident that one individual may be more or less careful than another, and that the test may yield different results in the hands of an equally experienced operator. However, it is believed that these results do give some tangible evidence of the extent of variation which may be expected when the test is performed by one who has had extensive experience with it. Although the specific decimal figures may not be directly applicable to the work of another individual of equal experi-

ence, the results afford a basis of judging the general quality of work. Any interpretation based on these data must be made with these limitations in mind and with due allowances for them.

INTERPRETATIVE VALUE OF THE DATA

In the Middle West the large centralized creameries have small cream stations within a radius of 500 miles of the plants. In these cream stations the operators buy, test, and ship the cream, which is brought in by the local dairymen. Even though field superintendents from the plants and representatives from the State dairy commissioner's office frequently check the fat tests of these operators, numerous cases of fraudulent tests are on record. It is in the checking of such fraudulent tests that data such as are presented in this paper may be interpreted to the best advantage.

For the sake of illustration, let it be supposed that a cream station operator had turned in an average of duplicate tests of 40.50 per cent on the same can of cream used in the first experiment. Let it be further supposed that the inspector (reader No. 2) got a test of 41.75 per cent fat on the can of cream. The chances are 30 to 1 that the inspector's average of 2 tests is not more than 0.390 from the "true" test or that the actual fat content of this can of cream is not outside the limits of 41.75 ± 0.39 (41.36 and 42.14). Since the station operator's test (40.50) is beyond these limits the inspector is justified in assuming that the station operator is in error. On the other hand, if the station operator's average of duplicate tests were 41.40 per cent, this value, being within the limits of normal variation of the inspector's work, would not be subject to his criticism.

The data in these experiments forcibly illustrate the necessity of making duplicate tests in order to enforce the stipulations of many of the State laws. In Kansas and in several other States, the laws regard any test as fraudulent if it is more than 1 per cent in error. This is interpreted to mean 1 per cent of the fat purchased and not a 1 per cent reading on the neck of the bottle. For example, in buying 100 pounds of cream containing 37 per cent fat, any test beyond the limits of 37 ± 0.37 would defraud the buyer or seller of more than 1 per cent of the fat purchased. In other words, the reading of the test in this case must be accurate within 0.37 of the true test to comply with the State law. The results obtained by reader No. 2 with the sour cream, which tested approximately 37 per cent, will be used to illustrate the fact that an inspector could not enforce this stipulation of the law if only single tests were used. The probable error of a single test on this cream was ± 0.169 , and 3.2 times this value establishes the limits of certainty at ± 0.54 per cent. In other words, the inspector has demonstrated that normal variation in his own work may account for an error as great as ± 0.54 in the reading of a single test.

If the cream station operator's test on this can of cream were 36.50 and a single test by the inspector were 37, he could not enforce the statutes, even though the disparity between tests exceeded the legal tolerance of 1 per cent of the fat purchased. In other words, the normal variation of the method is likely to exceed the stipulations of the statutes. In fact the data indicate that the normal variation of duplicate samples is just barely within the limits of the 1 per cent

tolerance. Again using the sour cream as an example (Table 2), this inspector has demonstrated that the average of duplicate tests may account for variations as great as ± 0.382 , which is almost identical with the statute limitations in testing 37 per cent cream. It is evident that in order to comply with a legal tolerance of 1 per cent variation in testing, duplicate determinations must be employed.

EFFECT OF CARELESS READING

The limits of accuracy of the Babcock method are so near the stipulations of many of the States' laws that precision in every step of the process is requisite. This is well illustrated in the highly variable readings of the tests reported by reader No. 6. (Table 2.) Although this man had had several years experience with the Babcock method and the manipulation of the test constituted one of his daily routine duties, his work was characterized by more speed than accuracy. When the probable error values for reader No. 6 (Table 2) are compared with those of the other readers it is seen that in nearly every case his variations are between wider extremes than are those of the other readers. The limits of variation for the most experienced readers (1 and 2) are just barely within the limits of accuracy demanded by the State laws. The errors resulting from less experience on the part of the other persons (3, 4, and 6) or lack of precision in reading are sufficient to render it doubtful whether they would always comply with the demands of the law.

TABLE 3.— *Distribution of 1,599 readings of 160 tests on sweet cream, and 160 tests on the same cream after souring*

SWEET CREAM									
Reader No.	Number of readings at per cent indicated								
	36	36.25	36.50	36.75	37	37.25	37.50	37.75	38
1.....			3	4	93	21	39		
2.....	1		1	9	120	11	18		
3.....	1	1	18	15	86	26	13		
4.....			4	18	76	48	14		
6.....	1	6	26	25	63	16	21	1	1
Total.....	3	7	52	71	438	122	105	1	1
SOUR CREAM									
1.....	1	1	16	21	89	20	12		
2.....		1	19	7	103	14	16		
3.....	1	2	46	30	64	16	2		
4.....	1	4	10	36	81	22	5	1	
6.....	5	10	65	22	54	9	4		
Total.....	8	18	145	116	391	81	39	1	
Total for sweet and sour cream.....	11	25	197	187	829	203	144	2	1

Table 3 shows the distribution of the 1,599 readings of 160 tests on sweet cream and 160 tests on the same cream after souring. A study of this table shows that most of the extreme readings on both sweet and sour cream were made by one reader (No. 6). The method

of tabulating the individual readings of the tests made it possible to check the work of each reader against that of other readers. In one case, for example, reader No. 6 reported a test of 36 per cent, whereas all the other readers recorded 37 per cent for the same bottle. It is evident in this case that the 36 per cent reading was erroneous and represents a variation due to inaccurate reading rather than to fluctuation in the test. In all cases where the extreme readings of 36, 37.75, and 38 per cent were reported, it was found that these were the result of erratic reading of a single reader, and did not represent the opinion of the other readers.

On examination of the data it was found that all but 2 of the 25 readings of 36.25 per cent were likewise the result of erroneous reading and did not conform to the readings of the majority. In other words, with 2 exceptions, the correct readings of the fat columns of the 320 tests should have been between the extremes of 36.50 and 37.50 per cent. Obviously, in calculating the error of the test all readings whether correct or not must be included, but in interpreting the actual variation of the fat columns in the necks of the bottles, elimination of apparently erroneous readings is justified.

TABLE 4.—*Variations in weighing sixteen 9-gm. samples of cream for the Babcock test*

[All weighings made by the same technician]

Weight of sample	Weight of sample	Weight of sample
<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
8.9922	8.9774	9.0044
8.9932	8.9800	8.9747
8.9998	9.0059	8.9872
8.9908	8.9641	9.0079
8.9802	8.9986	8.9939
8.9988		
	Mean..	8.9917

Standard deviation	0.0112	Probable error, duplicate weighings	±.0053
Coefficient of variability1245	3.2 × Probable error, single weighing	±.0240
Probable error, single weighing	±.0075	3.2 × Probable error, duplicate weighings	±.0169

ERROR IN WEIGHING THE SAMPLE

A certain amount of the variation in the results with the Babcock test is undoubtedly due to erroneous weighing. In order to measure the extent of this source of error 16 bottles were carefully weighed on analytical balances before and after admission of the sample. An important aspect of this experiment was that the technician who weighed all the samples was not aware of this check on his work. The results are given in Table 4. The extremes of the weighings of cream were 8.9641 and 9.0079 gm., with a mean of 8.9917 gm. Only 3 of the 16 weighings were in excess of 9 gm. The weighing most closely approximating 9 gm. was 8.9998 or 0.0002, and the most erroneous weighing was 8.9641 or -0.0359 gm. The values for 3.2 times the probable errors for single and duplicate weighings were +0.0240 and +0.0169 gm., respectively. That is to say, the technician in this case could be practically certain of weighing a single sample within +0.0240 gm. of the mean weighing (8.9677 to 9.0157 gm.). Similarly, he could be practically certain that the

average of duplicate weighings would not be more than ± 0.0169 gm. from the mean (8.9748 to 9.0086 gm.).

The error of 24 mgm. in weighing 37 per cent cream would cause a variation of 0.1 per cent (0.098) in the reading in the neck of the bottle. Since the limits of practical certainty of testing cream (based on single sample for reader No. 2) were found to be ± 0.54 per cent of fat, it follows that about one-fifth of his variation may be traceable to errors in weighing.

LIMITS OF VARIATION OF THE BABCOCK TEST WHEN UNKNOWN SAMPLES ARE TESTED.

In the experiments reported in Tables 1 and 2 the readers were aware of the fact that the tests were all made from the same can of cream and that the readings, therefore, should be essentially the same. It was recognized that this factor might reduce the error by minimizing the probability of large errors. In order to eliminate this factor, three lots of cream containing approximately 36, 37, and 37.75 per cent fat, respectively, were prepared, and identical samples of each submitted at different times to each of three laboratories. The numbering system employed and the close proximity of the fat tests gave the tester no clue to the identity of the sample being tested, although the technician was aware that his work was being checked. It should be mentioned that these samples were very carefully prepared by the method used in the other experiments, except that approximately 0.03 per cent formaldehyde was added, the screw-cap sample jars dipped in melted paraffin, and the samples kept at 35° to 40° F. until tested.

TABLE 5.—Frequency distribution of fat test readings on three samples of cream reported by three laboratories

SAMPLE NO. 1

		Number of readings at per cent indicated							
		35	35.5	36	36.5	37	37.5	38	38.5
1					11	35	3	13	
2		1	1	1	12	58	15	1	
3					8	80			
Total		4	1	1	31	173	18	14	

SAMPLE NO. 2

1			6	1	1		1	
2		2	1	8	3			
3			4	11	3			
Total		2	11	20	7	1		1

SAMPLE NO. 3

1					2	1	5	
2				1	2	7	5	3
3					1	1	14	
Total				1	5	9	24	3

TABLE 6.—Variation of fat tests on three samples of cream reported by three laboratories

SAMPLE NO. 1					
Laboratory No.	Tests	Extreme readings		Average reading	Limits of practical certainty in testing duplicate samples ^a
		Low	High		
	Number	Per cent	Per cent	Per cent	±
1	62	36.5	38.0	37.14	0.74
2	92	35.0	38.0	37.06	.80
3	88	36.5	37.0	36.95	.19
Total	242	35.0	38.0	37.04	.67

SAMPLE NO. 2					
1	10	35.5	38.0	36.05	1.25
2	14	35.0	36.5	35.93	.74
3	18	35.5	36.5	35.97	.48
Total	42	35.0	38.0	35.97	.80

SAMPLE NO. 3					
1	8	37.0	38.0	37.68	.64
2	18	36.5	38.5	37.69	1.12
3	16	37.0	38.0	37.90	.42
Total	42	36.5	38.5	37.77	.67

^a 3.2 times probable error of duplicate tests

Table 5 shows the distribution of the readings reported by the three laboratories on the three samples. In two of the laboratories (Nos. 1 and 2) some difficulty was experienced at first in testing the preserved samples, and although the tests were eliminated until the difficulty was overcome, it may account for part of the variation in the results of these two laboratories. Table 6, based on the data from Table 5, shows the degree of variation of the results to be considerably larger than was reported in Tables 1 and 2. It may be observed that the limits of a practical certainty in making duplicate tests of sample No. 1 were marked by ± 0.74 , ± 0.80 , and ± 0.19 per cent fat for each of the three laboratories. Although the average readings reported by the three laboratories were not so widely divergent, the extreme readings were very different. The results show that the error of testing may be greater than the preceding experiments would indicate.

TABLE 7.—Frequency distribution of fat test readings on 44 identical samples of cream ^a

[Work performed in routine manner at one laboratory]								
Number of readings at per cent indicated								
32.5	36.5	37.5	37.75	38.0	38.25	38.5	39.0	41.0
1	1	2	2	23	7	5	2	1

^a In this case the extreme low and high readings were 32.5 and 41, respectively, the average reading was 37.97, and the limit of practical certainty in testing duplicate samples ($3.2 \times$ probable error of duplicate tests) was ± 3.23 .

As previously mentioned, the technicians were aware that their work was being checked, and, no doubt, may have taken more care in the analyses than regular routine samples would have received. In order to measure the influence of this factor on the error of testing, 44 identical samples were prepared with the same care and precision as

used in mixing samples for the preceding experiments. These samples were sent to one laboratory, a few at a time along with a large number of other routine samples. In this case the tester not only was unaware of the identity of the samples but he was unaware of the fact that he was testing some identical samples in the routine of his day's work. The results reported in Table 7 are surprisingly variable.

It may be observed that the tests varied between the very wide extremes of 32.5 and 41 per cent fat, and that other wide variations were reported. This table also shows the limits of a practical certainty to be 3.23 per cent above or below the mean. Even though the results of this experiment were obtained under practical conditions and from a reputable laboratory, it is difficult to believe that they are representative of widespread conditions. The test of 32.5 per cent might easily have been the result of a 5 per cent error in reading the spread of the dividers on a 37.5 per cent test, but one can hardly explain the misreading of duplicate test bottles on this basis. Similarly, one can easily explain the 41 per cent reading by assuming a slip of the dividers over 3 points on a 38 per cent test, but the reports from this laboratory were supposedly made on the basis of the average of duplicate tests.

Whether or not the results are representative of routine testing, they do show that when check testing was done in the same laboratory (Table 6) much more careful work was reported. In other words, there is a marked tendency to pay less attention to precision in routine procedure than when one is aware that his work is being checked.

SUMMARY AND CONCLUSIONS

In this series of experiments an attempt was made to measure the limits of error of the Babcock test for cream. A large number of tests were made on a single can of sweet cream, and, in another trial, on cream before and after it had soured. The tests were read by several readers, both experienced and inexperienced. In one experiment identical samples of cream were sent to three laboratories for analysis. In another case samples were sent to one of these laboratories under conditions which prevented the operator from knowing that he was testing check samples.

The results of the first trial, consisting of 456 readings, indicate that the practical limits of variation of the test were 0.444 per cent. The second trial substantiated the results of the first, showing the limits to be 0.413 per cent on sweet cream and 0.443 on sour cream.

The extent of the error depended somewhat on the experience of the reader. However, the tendency of the reader to do precise work was found to be more important than experience.

The distribution of 1,599 readings on a can of sweet cream and the same cream after souring followed the normal curve with 97.5 per cent of the readings falling within approximately 0.5 per cent of the mean. The difference between the results obtained with sweet cream and sour cream was beyond the limits of normal variation of the 160 tests but was within the limits of normal variation when only duplicate tests were employed.

Cream samples which had been weighed into 9-gm. test bottles were reweighed on an analytical balance and found to check very closely to the correct weight. The error due to weighing was responsible for only about one-fifth of the total variation of the test.

The results obtained when submitting samples of known and unknown identity to three laboratories indicate that the error of routine testing is much greater when the operator is unaware that his work is being checked.

In the enforcement of laws pertaining to testing it is important that the limits of error of the test be taken into consideration by the inspector. These data indicate that in most cases an inspector can not be practically certain that a single test will be closer than 0.5 per cent to the correct test. When this normal variation in his own work is ignored in interpreting the disparity between the inspector's and station operator's tests, it may in some instances erroneously show the operator to be a violator of the law. An error of 0.5 per cent on the test on cream containing less than 50 per cent fat would introduce an error in excess of 1 per cent of the amount of fat purchased. This is in excess of the legal tolerance recognized by many States.

The results of these experiments, although not strictly applicable to other workers, show that the technician involved could not be certain that single tests on 37 and 40 per cent cream would be closer than ± 0.44 to ± 0.55 per cent fat, or that the average of duplicate tests would be closer than ± 0.31 to ± 0.39 per cent fat.

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GENETIC CHARACTERS IN RELATION TO CHROMOSOME NUMBERS IN A WHEAT SPECIES CROSS¹

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INTRODUCTION

Combined genetic and cytological studies of wheat species and hybrids between them are used for the information that they give concerning the phylogenetic relationships of the species. They are of interest from the standpoint of plant breeding also, since, in order to attack intelligently a breeding problem by the method of hybridization and selection, it is necessary to know the readiness with which the characters of the parents can be recombined in the progeny and the limitations involved. Species crosses have been used only to a limited extent as a means of obtaining improved varieties of wheat, and only a very few results of practical value have been secured. It is possible that much wider practical use could be made of such crosses if a more thorough knowledge were available concerning their genetic and cytological behavior.

REVIEW OF LITERATURE

The cultivated species of wheat (*Triticum* L.) on the basis of their chromosome numbers belong to three different groups—the einkorn group (*T. monococcum* L.) with 7 pairs of chromosomes, the emmer group (*T. durum* Desf., *T. polonicum* L., *T. dicoccum* Sch., and *T. turgidum* L.) with 14, and the vulgare group (*T. vulgare* Vill., *T. compactum* Host., and *T. spelta* L.) with 21 pairs. The various cytological results obtained in crosses between different species of the three groups and the amount of sterility in the hybrids have been used as an indication of the probable relationship between the species.

The chromosome behavior in pentaploid hybrids between the emmer and vulgare groups has been described by Kihara (7),³ Sax (11), Watkins (16), and Thompson (14). The hybrid in these crosses receives 14 chromosomes from one parent and 21 from the other. At the metaphase of the first reduction division of the F₁, 14 bivalents and 7 univalents are found, the 14 emmer chromosomes having paired presumably with 14 from the vulgare parent. The bivalents behave

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² Reference is made by number (italic) to Literature Cited, p. 178.

normally in both divisions. The univalents lag until the members of the bivalents have left the equatorial plate, then split equationally and go to the poles. In the second division, the univalents go to the poles at random. Some irregularities are found, such as the loss of univalents or the failure of univalents to split at the first division. On this basis, gametes formed by the F_1 would vary in the number of chromosomes from 14 to 21, 16, 17, and 18 being probably most frequent. Watkins (17) studied meiosis in the megaspore mother cells and came to the conclusion that the same course is followed in the reduction divisions of both the megaspore and microspore mother cells, the loss of chromosomes occurring about as frequently in one as in the other.

In the crosses of emmer and vulgare wheats, Sax (12, 13) found a striking association between chromosome numbers and morphological characters. A large proportion of the F_2 and F_3 segregates resemble one parent or the other, a preponderance resembling the emmer parent. In F_2 and following generations, the only fertile segregates are those having 14 chromosome pairs and resembling the emmer parent, or those with 21 pairs and resembling the vulgare parent. Some of the fertile segregates, however, may have one or two characters that are typical of the other type, but there is no evidence that the typical and desirable characters of the parents have ever been combined in a single variety of economic value.

The desirable characters include resistance to black stem rust, *Puccinia graminis tritici* Eriks. and Henn., and apparently Sax believes that it is quite improbable that a variety of the vulgare type could be obtained from such a cross with the rust resistance of the durum parent.

The report of Thompson (14) shows that the correlation between chromosome numbers and morphological characters is not so great as that suggested by Sax. In a cross of *Triticum vulgare* \times *T. durum*, he studied the inheritance of resistance to stem rust and 13 pairs of characters which distinguish *T. durum* from *T. vulgare*. His results agree with those of Sax except in a few instances. He obtained more intermediates in the F_2 and F_3 , although these tended to disappear in the F_3 and later generations. The plants with 14 pairs of chromosomes were of the durum type, and those with 21 pairs were of the vulgare type, but most plants of each type showed a few characters of the other type. A few vulgare plants were found resistant to stem rust, but to a lesser degree of resistance than that found in the durum parent. It would seem from this that any desired recombination of characters could be obtained.

In studies conducted with spring wheat using the genetic mode of attack, Hayes et al. (5) found that in crosses between a durum resistant to stem rust and a vulgare susceptible to it there was an indication of linkage of the durum characters and resistance. They show, however, that if large enough numbers are used, it is quite possible to get the desired combination of durum and vulgare characters. From one of these crosses between Marquis (*Triticum vulgare*) and Iumillo (*T. durum*) a fertile homozygous variety of wheat of the vulgare type has been obtained. This variety, Marquillo, a brief description of which is given by Hayes and Garber (4), has 21 pairs of chromosomes, is about equal to the vulgare parent in milling and baking qualities,

and has at least two genetic factors for stem-rust resistance which it inherited from its durum parent (6). It is susceptible to root rot, another character obtained from the durum parent. Hayes and Garber (4) also report some important results which have been obtained by McFadden in South Dakota. From a cross of Emmer (*T. dicoccum*) and Marquis, several wheats of the vulgare type highly resistant to stem rust were produced. Hope, one of these varieties, has not been tested sufficiently to determine whether it will be very valuable as a commercial variety. As stated by Clark and Ausemus (3) the immunity of Hope wheat from black stem rust and other diseases can be transmitted readily to selections from crosses with other vulgare varieties.

A. A. Sapehin (9) confirms the facts obtained by Hayes et al. In crosses between *Triticum vulgare* and *T. durum*, he found, as usual, a very complicated segregation of genetic characters. He obtained a large number of recombinations of the characters of both parents. Three of the vulgare types were resistant to Hessian fly, a character which they inherited from the durum parent. A most interesting type of plant obtained in this study from the cytological standpoint contained 36 chromosomes (16 bivalents and 4 univalents). This remained constant in chromosome number and morphological characters for a period of six years. This seems all the more remarkable since Kihara considered this a sterile combination. L. A. Sapehin (10) gives a report of the durum segregates of crosses of *T. durum* and *T. vulgare*. A cytological examination shows balanced sets of 28 chromosomes in these segregates, but the presence of so many vulgare characters indicates that, though the parental number has been retained, the sets differ qualitatively. In this work again is mentioned segregates resistant to Hessian fly and to drought.

In view of the seemingly conflicting results which have been obtained by different investigators, it is evident that much more work must be done with species crosses before the problem is solved entirely.

MATERIAL AND METHODS

The investigation reported in this paper was undertaken as a combined genetic and cytological problem to determine if possible the relationships between chromosome numbers and genetic characters in a cross between Velvet Don (*Triticum durum*) and Quality (*T. vulgare* Host.) and to throw more light on the feasibility of using such crosses in breeding improved varieties of wheat.

The cross was made in 1925, and the genetic and cytological studies of the F_1 , F_2 , and F_3 were carried on during the years 1926, 1927, and 1928, respectively. Cytological material of about 100 F_2 plants was examined, but definite chromosome counts and genetic results were obtained for only 24 of them. About 500 F_3 plants were examined in the same manner, results being secured for 68 of these.

Velvet Don has 14 pairs of chromosomes; a prominent keel extending the full length of the outer glume; a prominent collar which is a structure extending completely around most of the stems at the base of the lowest spikelet; a solid stem in the internode just below the spike; long, narrow glumes; a rachis internode length of about 3.6 mm.; long awns; heavily pubescent glumes; and red seed. It is highly resistant under field conditions to stem rust (*Puccinia graminis tritici*) and to leaf rust (*P. triticea* Eriks.).

Quality, on the other hand, has 21 pair of chromosomes; a less pronounced keel extending only part of the length of the glume; collar absent or extending only part way around the stem; hollow stem; shorter and wider glumes than are found in Velvet Don; an internode length of about 6 mm., tip awns, glabrous glumes, and white seed. It is susceptible to both stem and leaf rust in the field.

Characters such as chromosome number, keel form, collar, stem cavity, glume shape, and internode length, as contrasted in Velvet Don and Quality, distinguish nearly all durum from vulgare varieties; the other characters are common to both species.

The seed for the F_1 of this cross was turned over to the writer by Dr. H. K. Hayes of the Minnesota Agricultural Experiment Station. The F_1 plants were grown in an isolated plot to prevent crossing with other wheats. The F_2 lines were grown in the plant pathology nursery at University Farm, St. Paul, Minn., where they were subjected to an artificial epidemic of stem rust. A heavy leaf rust also was obtained on the F_2 lines, although quite by accident. Evidently the spray used to induce the stem-rust epidemic carried leaf-rust spores as well. Rust notes were taken on individual plants in the field. Notes on other plant characters were determined on a single head of each individual plant in the laboratory. To obtain the rachis internode length, 10 internodes, beginning with the second from the base, were measured in millimeters. The average of these 10 was taken as the index of internode length. The percentage of seed set as an indication of sterility was taken on the F_1 plants by counting the total number of heads in a line, obtaining the average number of spikelets per head and the total number of seeds produced. Two seeds per spikelet were used as 100 per cent fertility, and the percentage of seed set was calculated on that basis. In the F_2 data, the plants were placed in five groups on the basis of their fertility. The plants in Group 1 were completely fertile, those of Group 2 about 75 per cent fertile, Group 3 about 50 per cent, Group 4 about 25 per cent, while Group 5 was completely sterile. A few plants were designated 5- to indicate almost complete sterility.

It was part of the plan to make a study of the rust reactions in the seedling stage of the segregates of each of the generations in the greenhouse. As a preliminary to such a study, the parents, Velvet Don and Quality, were tested in the greenhouse in the winter of 1925-26. Although Velvet Don has been known to be highly resistant to stem rust in the field for 20 years, it was found to be very susceptible in the seedling stage in these tests. Both Velvet Don and Quality were inoculated with 14 different physiologic forms of stem rust. Velvet Don was found to be susceptible to all of them, but Quality showed some resistance to forms Nos. 10, 19, 23, 27, and 38. (Table 1.)

TABLE 1.—The reaction to stem rust of wheat, *Puccinia graminis tritici*, of Velvet Don and Quality in the seedling stage in the greenhouse

[In this table, the symbol R stands for resistant, MR moderately resistant, MS moderately susceptible, and S susceptible]

Parent	Reaction to rust form No. —													
	1	10	15	18	19	21	23	27	29	33	36	38	40	41
Velvet Don...	S	S	S	S	S	S	S	S	S	MS	S	S	S	S
Quality.....	S	MR	S	S	R	S	MR	MR	S	MS	S	MR	S	S

From these tests, it was evident that no segregation could be expected in reaction to many of the physiologic forms in the seedling stage in the greenhouse, since both of the parents were susceptible to many of the forms. The rust tests were made, therefore, in the field, and in order that the readings might be comparable all plantings were made on the same date. This was a disadvantage from the standpoint of the cytological studies, since it gave only a short period of time in which good material could be obtained.

Chromosome counts were made in the meiotic divisions of the pollen mother cells. Belling's (2) iron-acetocarmine method was used to examine the anthers in order to obtain the right stages. The anthers found to be in the proper stage of development were fixed in Allen's (1) modified Bouin's solution. The usual method of making permanent slides for such material was followed. The sections were cut from 10μ to 15μ thick, those with a thickness of 12.5μ giving the best results. A few of the slides were stained with Haidenhain's iron-alum haematoxylin, but for most of them a modified triple stain combination was used. By this method, a nice contrast was obtained between the chromatin material and the cytoplasm by staining in a 1 per cent aqueous solution of safranin for one hour or more. If chromosome counts only are required, the gentian violet and orange G of the triple stain may be omitted, which is an advantage from the standpoint of time and expense.

Chromosome counts were made in the metaphase or early anaphase of the first division. The number of bivalents and univalents were obtained, and in most cases the number of univalents were checked in the side view of the metaphase.

GENETIC AND CYTOLOGIC DATA

Only a few chromosome counts were made of the parents of the Velvet Don \times Quality cross, but these were enough to verify the fact that Velvet Don cytologically was a typical durum with 14 pairs of chromosomes behaving regularly, and Quality was a vulgare with 21 pairs. The characters of each of the parents have been described in Material and Methods.

The F_1 , as has been reported before for such crosses, has a total of 21 chromosomes in the metaphase of the first division—14 bivalents and 7 univalents. This was verified by the total of 35 found in the early anaphase of the first division before the univalents had split. The F_1 plants had a keel and collar similar to the durum, although there were frequent variations resulting in an intermediate condition for these characters. The stem cavity and glume shape were intermediate between the parents. The pubescence and red seed color of the Velvet Don were dominant. The beards were intermediate. The F_1 plants were not artificially inoculated to obtain an epidemic of stem rust, but a trace to 5 per cent of rust was observed, the shape and size of the pustules clearly indicating susceptibility.

The F_1 plants were spaced about 6 inches apart in the field, to give an opportunity for tillering. One hundred and forty-seven plants were grown, and these produced 1,278 heads, or on the average about 8.7 heads per plant. Fifteen hundred and eighty-four seeds were harvested. On the basis that there were on the average

12.5 spikelets per spike, and that two seeds per spikelet constitute perfect seed setting, the F_1 plants set seed to the extent of about 5 per cent.

The chromosome counts and genetic results obtained for the parents, F_1 and F_2 are given in Table 2.

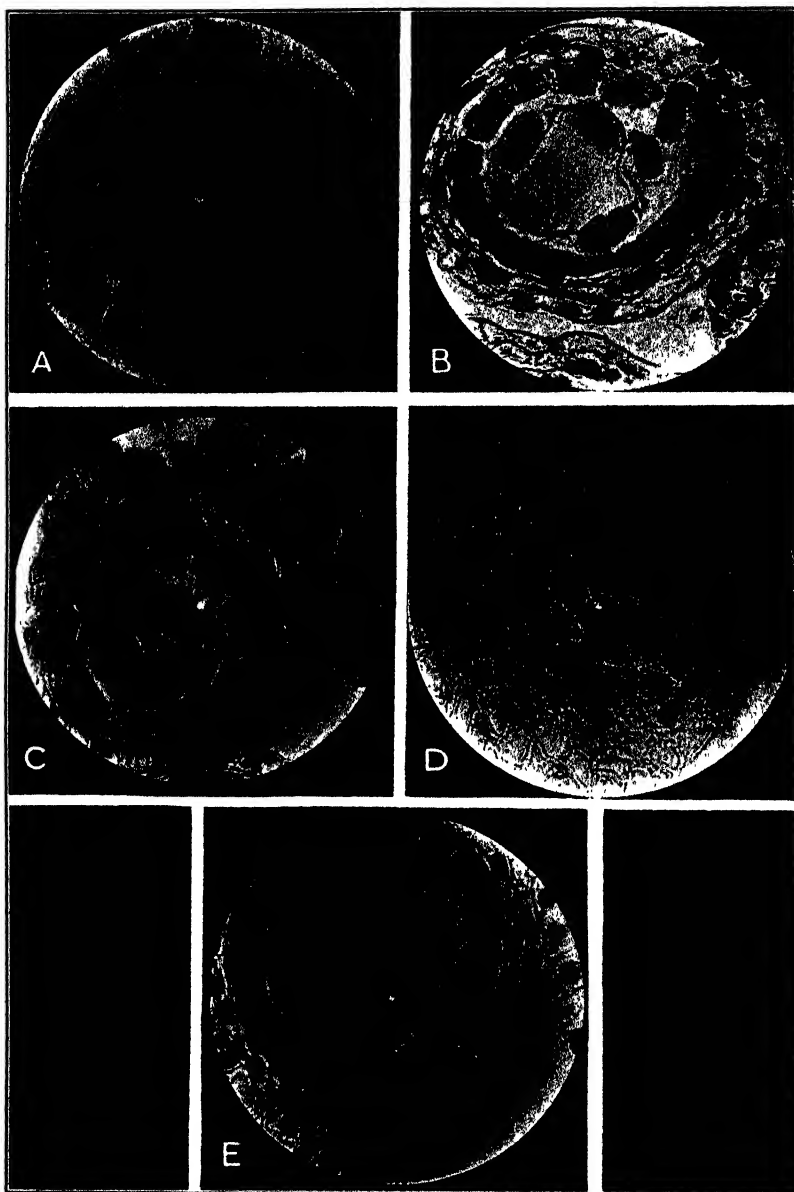
TABLE 2.—Chromosome numbers and characters of parents, F_1 and F_2 plants of the Velvet Don×Quality wheat cross ^a

Plant No.	Somatic No.	Meta-phase No.	Keel	Collar	Stem cavity	Glume shape	Beards	Pubescence	Seed color
Velvet Don P ₁	28	14 _{II}	D	D	D	D	b	P	R
Quality P ₁	42	21 _{II}	V	V	V	V	B	p	r
F_1	35	14 _{II} -7 _I	D	D	I	I	I	P	R
F_2									
567-3.....	42	21 _{II}	D	D	V	D	B	p	R
567-6.....	38	17 _{II} -4 _I	D	V	V	V	B	P	R
567-7.....	26	14 _{II}	D	V	I	V	I	P	
567-8.....	26	14 _{II}	D	D	D	D	B	P	R
568-1.....	29	14 _{II} -1 _I	V	D	V	V	I	p	r
569-2.....	42	21 _{II}	D	D	V	D	I	p	R
570-5.....	42	21 _{II}	D	V	I	D	I	P	R
571-1.....			V	V	V	V	B	P	r
571-5.....	30	14 _{II} -2 _I	D	D	V	D	I	P	R
571-6.....	29	14 _{II} -1 _I	D	D	D	I	B	p	R
571-9.....	42	21 _{II}	D	D	I	I	b	P	R
571-12.....	30	14 _{II} -1 _I	D	D	D	D	B	P	R
571-14.....	29	14 _{II} -1 _I	D	D	D	D	I	P	R
572-4.....	28	14 _{II}	D	D	D	D	I	p	r
572-12.....	28	14 _{II}	D	D	I	D	I	P	R
572-14.....	42	21 _{II}	D	D	V	D	B	P	r
574-5.....	32	15 _{II} -2 _I	D	D	D	D	B	P	
575-5.....	28	14 _{II}	V	V	D	V	B	p	
576-2.....	28	14 _{II}	D	D	D	D	I	P	R
577-3.....	28	14 _{II}	D	D	D	V	b	p	R
577-4.....	28	14 _{II}	D	D	D	I	I	P	R
579-4.....	28	14 _{II}	D	D	V	D	R	P	R
580-4.....	35	14 _{II} -7 _I	D	D	V	I	B	P	R
582-6.....	28	14 _{II}	D	D	D	D	I	P	R
583-3.....	28	14 _{II}	D	D	D	D	B	P	r
583-7.....	28	14 _{II}	V	V	V	V	b	p	

^a Symbols used in tables: II, bivalents; I, univalents; D, durumlike, V, vulgarelike, I, intermediate; B, beardless or tip awned as the vulgare parent; b, bearded; P, pubescent; p, glabrous; R, red, r, white

Five F_2 plants had 42 chromosomes as the somatic number. One had 38; 1, 35; 1, 32; 2, 30; 3, 29; and 11, 28.

Three of the 42-chromosome plants had as many as 3 of the 4 differentiating characters—keel, collar, stem cavity, and glume shape—inherited from the durum parent. The other 2 plants of this group had 2 such characters. The 38-chromosome plant had a durum keel, but vulgare collar, stem cavity, and glume shape. The plant with 35 chromosomes had a durum keel and collar, with a vulgare stem cavity and an intermediate glume shape. The plant with 32 chromosomes was durumlike in the four differentiating characters. Plant 571-5 with 30 chromosomes was durumlike in keel, collar, and glume shape, but had a vulgarelike stem cavity, while the other 30-chromosome plant, 571-12, had all four of these characters durumlike. Two of the three 29-chromosome plants were durumlike in all 4 of these characters, but the other one had durum keel, collar, and stem cavity, with a vulgare glume shape. The 28-chromosome plants showed a somewhat greater association between chromosome number and durum characters than did those with 42. Four of the 28-chromosome group had all 4 of the differentiating characters durumlike,



PHOTOMICROGRAPHS OF POLLEN MOTHER CELLS IN MEIOTIC DIVISION

A, Metaphase side view of the first division of the F_1 plant No. 7-1 showing two lagging univalents, one on each side of the plate; B, metaphase polar view of the first division of the F_1 plant 32-14 showing 14 bivalents; C, metaphase polar view of the first division of another F_1 plant showing 14 bivalents on the equatorial plate; D, anaphase side view of the completely sterile F_1 plant 21-35 which had 39 chromosomes, 18 bivalents, and 3 univalents; the photomicrograph shows 3 unpaired univalents just after they have split equatorially (same cell as is shown in Fig. 1, G); E, anaphase of the first division of a 14-chromosome plant showing the characteristic shape of the chromosomes.

4 others had 3 such characters, 2 had 1, and 1 was vulgarelike for all 4 of the characters—keel, collar, stem cavity, and glume shape.

On the basis of chromosome numbers, it is seen that the results obtained are similar to what has been described by other workers for such crosses; that is, more 28's and 42's and fewer intermediates than would be expected if gametes with from 14 to 21 chromosomes were formed in the theoretical ratio, and a random mating of these gametes took place in the production of viable zygotes.

In a number of the F_2 plants, frequent end-to-end pairing of some of the bivalents was observed. Trisomes and tetrasomes were also found.

Cytological material of a large number of plants of the F_3 lines was examined, and notes were taken on the morphological character of each one. Chromosome counts and genetic characters were obtained on 68 plants, and this report will include only the results from these. The progeny of the 42-chromosome plants were studied first, as the vulgare type is of more interest to the plant breeder. The F_3 line No. 7 is the progeny of a 42-chromosome plant. The data on chromosome numbers and genetic characters are given in Table 3.

TABLE 3.—*Chromosome numbers and characters in an F_2 plant (567-3) and the F_3 line derived from it*

Plant No.	Somatic No.	Keel	Collar	Stem cavity	Glume shape	Beards	Pubes- cence	Seed color	Leaf rust	Stem rust	Inter- node length	Ster- ility
											Mm.	
F_2 567-3	42	D	D	V	D	b	p	R				
F_3												
7-1	42	V	V	V	V	b	p	R	80S	^a 25S	7.5	2
7-5	42	D	D	V	D	b	p	R	85S	T	7.0	1
7-12	42	V	V	V	V	b	p	R	60S	5R	7.1	1
7-15	42	D	V	V	V	b	p	R	80S	30S	8.0	2
7-16	42	D	D	V	D	b	p	R	70S	5R	6.8	3
7-17	42	V	V	V	V	b	p	R	80S	5R	8.2	2
7-27	42	D	D	V	D	b	p	r	80S	10R	7.4	3
7-28	42	D	D	V	D	b	p	r	80S	5R	5.1	1
7-32	42	V	V	V	V	b	p	R	80S	10S	6.8	1
7-34	42	D	D	V	D	b	p	R	65S	20S	7.5	2
7-39	42	D	V	V	V	b	p	r	80S	30S	7.1	1
7-40	42	D	V	V	V	b	p	r	60S	30S	6.4	1
7-41	42	V	V	V	D	b	p	R	80S	20S	6.1	1
Velvet Don check	28	D	D	D	D	b	p	r	7R	2R	3.6	1
Quality check	42	V	V	V	V	B	p	r	81S	71S	6.0	1

^a Symbols used.—With reference to leaf or stem rust: T, trace; R, resistant; SR, semiresistant; S, susceptible. The numbers give the percentage of rust found. For other symbols used see footnote a, Table 2.

^b Numbers indicate degrees of sterility as follows: 1, completely fertile; 2, about 75 per cent fertile; 3, about 50 per cent fertile; 4, about 25 per cent fertile; 5—, almost completely sterile; 5, completely sterile.

Thirteen plants of this line were all in the 42-chromosome group. This would indicate that the line is uniform for chromosome number and might be expected to remain constant in later generations. Univalents were found occasionally, but not more frequently than in the vulgare parent. A photomicrograph of a pollen mother cell of plant No. 7-1 (pl. 1, A) shows the bivalents grouped on the equatorial plate with two univalents, one on either side of the plate.

With only 13 plants to choose from, the data show some interesting combinations of durum and vulgare characters. The F_2 parent plant resembled Velvet Don in keel, collar, glume shape, beards, and seed color, and Quality in stem cavity and pubescence. Segregation, as shown by the F_3 line, took place for keel, collar, glume

shape, and seed color. Twelve of the plants had hollow stem cavities such as are found in the vulgare parent. One was classified intermediate, but this may have been a vulgare also with slightly thicker culm walls than is usually found in the vulgare types. The F_2 parent plant was bearded and should have bred true for this character. One F_3 plant, 7-12, was classified as intermediate for beards, but this may have been caused by field hybridization. All the plants of the line studied were susceptible to leaf rust, but their reaction to stem rust varied from very resistant to susceptible, although the highest percentage of stem rust found on them was less than half of that found on Quality, the susceptible P_1 . The internode lengths of all these plants were comparable to that of the vulgare parent. No very high correlation seemed to exist between the 42-chromosome number and the vulgare characters, although considered as a whole the vulgare characters outnumbered the durum. Some association between stem-rust resistance and distinctive durum characters is seen in the fact that four of the plants highly resistant to stem rust had three typical durum characters each. Despite this, No. 7-17 is a plant in which is combined the chromosome number and characters of the vulgare parent and the stem-rust resistance of the durum P_1 . This plant was very fertile, showing about 75 per cent seed setting. Plant 7-12 is another vulgare type which combines the stem-rust resistance and part of the leaf-rust resistance of the durum with the typical vulgare characters. It is especially interesting from the standpoint of plant breeding that, in a very small number of plants, such combinations can be obtained. If a progeny test in later generations shows these to be constant for chromosome numbers and homozygous for botanical characters, they represent recombinations which the plant breeder would consider desirable.

Lines 12 and 13 are the progeny of the F_2 plant 569-1. The data for chromosome numbers and characters of these lines are given in Table 4.

TABLE 4.—Characters of an F_2 plant (569-1) and chromosomes and characters of the F_3 line derived from it^a

Plant No.	So-matic No.	Keel	Col-lar	Stem-cav-ity	Glume-shape	Beards	Pu-bes-cence	Seed color	Leaf rust	Stem rust	Inter-node length	Ster-ility
											Mm.	
F_2 : 569-1		V	D	V	V	I	p	r				
F_3 : 12-1	42	V	V	V	V	B	p	R	85S	60S	5.3	2
13-1	42	V	V	I	V	I	p	R	5R	60S	5.7	3
13-4	42	V	D	V	V	I	p	R	85S	70S	6.8	1
13-6	42	V	V	V	V	B	p	r	85S	70S	6.3	3
13-9	42	V	V	V	I	I	p	R	70S	15R	6.5	2
13-12	42	V	V	V	V	I	p	R	70S	70S	5.6	2
13-20	42	V	D	V	V	B	p	r	80S	40S	7.0	1
13-21	42	V	D	I	I	I	p	r	70S	50S	4.4	3
13-23	42	V	D	V	V	B	p	r	85S	5R	6.4	4
13-27	42	V	V	V	V	B	p	R	80S	5R	4.6	2
13-29	42	V	V	V	V	B	p	r	85S	15SR	6.7	2
13-32	42	V	V	V	V	I	p	R	75S	60S	6.0	3
Velvet Don check	28	D	D	D	D	b	p	R	7R	2R	3.6	1
Quality check	42	V	V	V	V	B	p	r	81S	71S	6.0	1

^a For explanation of symbols see footnote a, Table 2, and footnotes a and b, Table 3.

No reliable chromosome count was obtained on 569-1, but it had the appearance of a *vulgare*. The F_3 data show that it was probably a plant having 21 pairs of chromosomes, since all 12 F_3 plants were found to be in that class. From the F_2 data the F_2 parent should have been classified as red for seed color since a segregation occurred, and from the F_1 that red seed color was dominant. This is an error which is easily made in classifying the seed of species hybrids for color, because the shriveled condition of the seeds of some plants makes such classification very difficult. The results here emphasize again the importance of a progeny test to verify an F_2 classification. Three of the four characters differentiating the two species were of the *vulgare* type in the F_2 parent of lines 12 and 13. As a consequence, the F_3 plants were uniform for the *vulgare* type of keel, stem cavity, and glume shape. One of the plants, 13-21, was classified as *durum* for stem cavity, but irregularities of this nature occasionally occur.

The segregates of lines 12 and 13 varied in their reaction to stem rust from 5R to 70S. From this F_3 line, it would be possible to obtain again *vulgare*-like segregates with 42 chromosomes and the stem rust resistance of the *durum* P_1 . In no case so far discussed has resistance to both stem and leaf rust been found in the same plant. Plant No. 13-1, however, is *vulgare*-like in most of its characters and is as resistant to leaf rust as Velvet Don. A progeny test of this plant will have to be made to determine the constancy for the different characters, especially for its leaf rust reaction.

The F_3 line No. 9 was variable for all the characters studied except seed color. Table 5 gives the data for chromosome numbers and genetic characters for this line.

TABLE 5. - *Chromosome numbers and characters in an F_2 plant (567-6) and the F_3 line derived from it*

Plant No.	Somatic No.	Metaphase No.	Keel	Color	Stem cavity	Glume shape	Beards	Pubes- cence	Seed color	Leaf rust	Stem rust	Inter- node length	Ster- ility
												Mm	
F_2 567-6	38	17 ₁₁ -4 ₁	D	V	V	V	B	P	R				
F_3													
9-19	36	16 ₁₁ -4 ₁	D	D	V	V	B	p	R	50S	T	4.8	2
9-26	38	17 ₁₁ -4 ₁	D	D	I	V	I	P	R	80S	20SR	5.0	4
9-28	33	15 ₁₁ -3 ₁	D	D	V	D	R	P	R	60S	20S	5.4	3
9-32	34	15 ₁₁ -4 ₁	D	V	V	V	I	P	R	40S	60S	5.3	5-
9-36	42	21 ₁₁	V	V	V	V	B	p	R	80S	70S	5.3	2

^a For explanation of symbols see footnote a, Table 2, and footnotes a and b, Table 3.

The F_2 plant No. 567-6, from which this line was grown, had approximately 38 chromosomes. From two to six univalents were observed in side views of the metaphase of the first division. The behavior of the chromosomes was very irregular. Many end-to-and bivalents were observed as well as many trisomes. Other irregular combinations were frequent. A polar view of a pollen mother cell in the metaphase of the first division is shown in Figure 1, A. The F_3 plants, 9-19, 9-26, 9-28, and 9-32, were as difficult to study as the parent, and the counts in all these are only approximate. Plants 9-19 and 9-28 were quite similar to the parent in chromosome behavior. Trisomes occurred frequently and occasionally a tetrasome was found.

Plant 9-32 with 34 chromosomes was almost completely sterile, only two or three shriveled seeds being found in it. A polar view of an anaphase of the first division of this plant is shown in Figure 1 B.

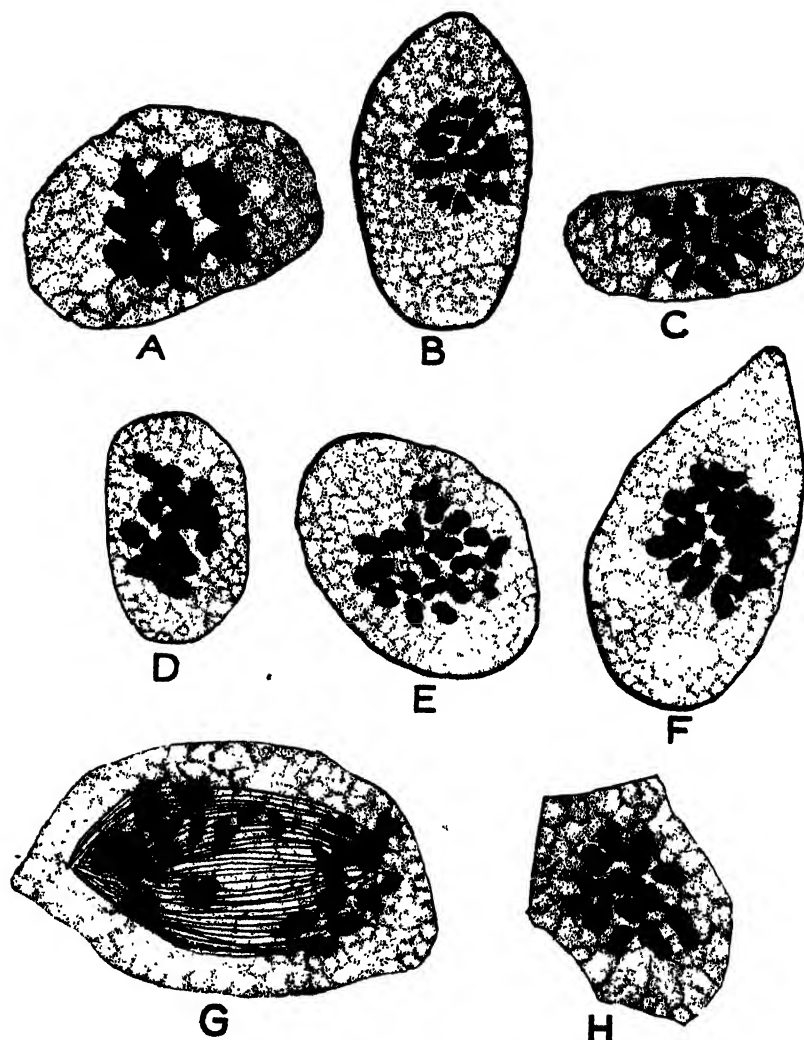


FIGURE 1.—Pollen mother cells in meiotic division. The figures were drawn from single sections, a camera lucida being used. A, Metaphase polar view of the first division of the F_2 plant 567-6 showing about 17 bivalents and 4 univalents. B, Anaphase polar view in the F_3 plant 9-32 which had 34 chromosomes, the drawing showing one group of 17. C, Metaphase polar view of the first division of the F_3 plant 571-14 which had 14 bivalents and 1 univalent. D, Metaphase polar view of the F_3 plant 34-14 which had 14 bivalents and was almost completely sterile. E, Metaphase polar view of the first division of the F_3 plant 7-28 showing 21 bivalents; this plant had the stem-rust resistance of the durum parent. F, Metaphase polar view of the first division of the F_3 plant 13-1 showing 21 bivalents; this plant had the leaf-rust resistance of the durum parent. G, Anaphase side view of the completely sterile F_3 plant 21-35 which had 39 chromosomes with 18 bivalents and 3 univalents; the drawing shows 3 unpaired univalents just after they have split equationally. H, Metaphase polar view of the first division of the F_3 plant 56-18 showing 14 bivalents; this plant had the stem-rust susceptibility of the vulgare parent.

The data for the F_2 plant 571-1 and its F_3 progeny are given in Table 6.

TABLE 6.—*Characters of an F₂ plant (571-1) and chromosomes and characters of the F₃ line derived from it*^a

Plant No.	So-matic No.	Meta-phase No	Keel	Col-lar	Stem cav-ity	Glume shape	Beards	Pu-bes-cence	Seed color	Leaf rust	Stem rust	Inter-node length	Ster-ility
												Mm.	
F ₂ : 571-1			V	V	V	V	B	P	r				
F ₃ :													
21-5	29	14 ₁₁₋₁₁	V	V	D	V	B	P	r	20SR-S	15S	4.4	2
21-7	28	14 ₁₁	V	D	D	D	B	P	R	10R	T	3.5	2
21-22	28	14 ₁₁	D	D	D	D	I	P	R	70S	40S	4.0	3
21-23	30	14 ₁₁₋₂₁	V	D	D	D	B	P	R	10R	5R	3.5	4
21-25	42	21 ₁₁	V	D	V	V	B	P	R	75S	40S	4.6	4
21-35	39	18 ₁₁₋₃₁	D	D	D	D	B	P			T	5.3	5
21-36	35	14 ₁₁₋₇₁	D	V	I	V	I	P	R	80S	50S	5.3	3
21-40	28	14 ₁₁	D	D	D	D	B	P	R	20R-SR	T	3.8	3

^a For explanation of symbols used see footnote a, Table 2 and footnotes a and b, Table 3.

The F₂ parent of line 21 was classified as vulgare, but a reliable chromosome count was not obtained. The F₃ results lead to the conclusion that it had a high chromosome count, either 42 or approaching 42 in number. A large F₃ line was raised from it, showing that it was a fairly fertile combination. The segregations for genetic characters as found in the F₃ line are difficult to account for. There is the possibility that the F₂ was incorrectly classified for several of its characters. It seems certain, however, that the characters were more vulgare-like than durum. One plant, No. 21-35, is of special interest. It contained 39 chromosomes. The univalents varied from three to seven. Three univalents in the process of division are shown in Figure 1, G. Plate 1, D, is a photomicrograph of the same pollen mother cell. Although this plant belonged in the higher group from the standpoint of chromosome numbers, it had the durum type of character for keel, collar, stem cavity, glume shape, and stem-rust resistance. It was, however, completely sterile and is thus eliminated from further tests.

The breeding behavior of a plant which, from counts made in the anaphase of the first division, had a total of 30 chromosomes is shown in Table 7.

 TABLE 7.—*Chromosome numbers and characters in an F₂ plant (571-12) and the F₃ line derived from it*^a

Plant No.	So-matic No.	Keel	Col-lar	Stem cav-ity	Glume shape	Beards	Pu-bes-cence	Seed color	Leaf rust	Stem rust	Inter-node length	Ster-ility
											Mm.	
F ₂ : 571-12	30	D	D	D	D	B	P	R				
F ₃ :												
32-6	28	D	D	D	D	R	P	R	5R	T	3.4	3
32-9	28	D	D	D	D	R	P	R	5R	60S	3.3	4
32-14	28	D	D	D	D	R	P	R	5R	60S	2.8	2
32-16	28	D	D	D	D	b	P	R	5R	30S	3.3	4
32-17	28	D	D	D	D	B	P	R	5S	20S	3.8	3
32-19	28	D	D	D	D	B	P	R	5R	T	4.1	2
32-23	28	D	D	D	D	B	P	R	5R	T	3.8	3
32-34	28	D	D	D	D	B	P	r	5R	60S	3.8	1
32-35	28	D	D	D	D	b	P	r	5R	10S	3.7	3
32-39	28	D	D	D	D	B	P	r	5R	5R	4.3	3

^a For explanation of symbols see footnote a, Table 2 and footnotes a and b, Table 3.

All of the plants of the F_3 line 32 had 14 pairs of chromosomes in the metaphase of the first division. This indicates that the gametes with 14 chromosomes produced by this parent plant were either more numerous or more effective in the fertilization process. Slight irregularities in pairing were found in these plants, but on the average they were similar to the durum parent. The F_2 parent was durum-like from the standpoint of its morphological characters of keel, collar, stem cavity, and glume shape. All the plants of the F_3 line were of the same type for these four characters. The internode length also showed them to belong to the durum class. Segregation occurred for beards, pubescence, and seed color. Nine of the ten plants gave a leaf rust reaction of 5R, the other one of 5S. The reaction for stem rust varied from a trace to 60S, showing a range from the resistance of the Velvet Don to almost the susceptibility of the Quality. The association of chromosome numbers and durum characters with stem-rust resistance is not, apparently, very great in this line, since there were three plants nearly as susceptible as the vulgare P_1 , and only four with the resistance of the durum P_1 . In this case, the susceptibility of the vulgare parent has been found in plants with the chromosome number and differentiating morphological characters of the durum parent.

The data for chromosome numbers and characters of a 29-chromosome plant, 571-14, and its F_3 progeny are given in Table 8.

TABLE 8.—Chromosome numbers and characters in an F_2 plant (571-14) and the F_3 line derived from it ^a

Plant No	Somatic No.	Keel	Collar	Stem cavity	Glume shape	Beards	Pubescence	Seed color	Leaf rust	Stem rust	Internode length	Sterility
											Mm	
F_2 571-14.....	29	D	D	D	D	I	P	R				
F_3												
34-1.....	28	D	D	D	D	B	P	R	10SR-S	30SR	3.5	2
34-2.....	28	D	D	D	D	B	p	r	10S	50S	3.1	5-
34-4.....	28	D	D	D	D	B	P	R	5SR	5R	3.4	3
34-5.....	28	D	D	D	D	B	P	R	15SR		3.5	5-
34-6.....	28	D	D	D	D	I	P	R	15SR	20SR	3.4	5-
34-7.....	28	D	D	D	D	B	P	R	20R-S	5R	3.2	3
34-8.....	28	D	D	D	D	B	P	R	20S	50S	3.2	2
34-9.....	28	D	D	D	D	B	P	R	30S	5R	3.8	2
34-12.....	28	D	D	D	D	B	P	R	20SR-S	60S	3.5	3
34-14.....	28	D	D	D	D	B	p	R	15SR	10SR	2.7	5-
34-15.....	28	D	D	D	D	b	p		20SR	30S	3.1	5
34-16.....	28	D	D	D	D	B	p	R	20SR	60S	3.8	2

^a For explanation of symbols see footnote a, Table 2, and footnotes a and b, Table 3.

The F_2 plant, 571-14, had 29 chromosomes. The pairing in this plant was very irregular. Tetrasomes were found frequently. A pollen mother cell in the metaphase of the first division with 15 chromosomes is shown in Figure 1, C. This plant bred true for keel, collar, stem cavity, and glume shape. From the standpoint of the differentiating characters and internode length, all 12 plants in this line would be classified as durum. These plants all have intermediate types of reaction to leaf rust. Although there is variation, none of them shows the resistance to leaf rust of the durum parent. About the same variation is found in the reaction to stem rust of the various plants of this line as was found in line 32, with little association of stem-rust resistance and durum characters.

The most interesting feature of line 34 is the amount of sterility found in some of the plants. One plant showed complete sterility and four others were almost completely sterile, producing only a very few shriveled seeds. This is a good illustration of the importance of chromosome quality rather than numbers, and the result of incompatibility of chromosomes which show enough affinity for each other, even to form pairs. Lines 32 and 34 are the progeny of plants which Kihara (7) designated as belonging to fertile combinations of the "decreasing group." The plants of this group decrease in chromosome numbers in later generations until the somatic number 28 of the durum P_1 is reached. These two lines and their parents furnish additional evidence as proof of this hypothesis.

Two F_3 lines were grown from plants having 14 pairs of chromosomes and classified as durum for the characters of the keel, collar, stem cavity, and glume shape. Chromosome counts and genetic data were obtained on eight of the F_3 plants. (Table 9.)

TABLE 9.—*Chromosome numbers and characters in two F_2 plants (572-4 and 576-2) and the F_3 lines derived from them*^a

Plant No	Somatic No	Keel	Collar	Stem cavity	Glume shape	Beards	Pubescence	Seed color	Leaf rust	Stem rust	Internode length	Sterility
											Mm	
F_2 572-4	28	D	D	D	D	i	p	r				
F_3												
38-1	28	D	D	D	D	B	P	R	25SR	10SR	4 0	3
38-7	28	D	D	D	D	B	P	R	5R	T	3.6	2
38-8	28	D	D	D	D	B	p	R	10S	T	4 4	3
F_2 576-2	28	D	D	D	D	I	P	R				
F_3												
56-2	28	D	D	D	D	b	P	r	5R	40S	3 2	2
56-5	28	D	D	D	D	B	P	R	10SR	40S	3 2	2
56-6	28	D	D	D	D	B	P	R	5R	60S	4 0	3
56-18	28	D	D	D	D	B	P	r	5R	20SR	3.4	2
56-20	28	D	D	D	D	B	P	R	20R-SR	30S	3.1	4
56-27	28	D	D	D	D	B	P	R				

^a For explanation of symbols see footnote a, Table 2, and footnotes a and b, Table 3.

^b No head matured.

All of these plants had 14 pairs of chromosomes, and all had the characteristic durum type of keel, collar, stem cavity, glume shape, and internode length. As a result, all of them would be classified as durum plants. The F_2 plant, 572-4, may have been classified incorrectly for pubescence and seed color as indicated by its breeding in the F_3 . There is the possibility that three F_3 plants resulted from natural crosses with a 14-chromosome male parent. All eight of the plants of these two lines showed an intermediate amount of fertility, only one of them, 56-27, showing as low as 25 per cent seed setting. Four out of the eight plants showed the Velvet Don reaction to leaf rust, but none of them was as susceptible as Quality, although some were intermediate to the parents.

The reaction to stem rust varied from 5R, which is similar to Velvet Don, to 60S, which is nearly as susceptible as Quality. Such a wide variation found in so few plants does not suggest a very close association between chromosome number, typical durum characters, and manner of reaction to leaf and to stem rust.

The primary purpose of this study was to determine, if possible, the relationships between chromosome numbers and genetic characters in a cross between a durum variety of wheat with 14 pairs of chromosomes and a vulgare variety with 21 pairs. The phase of the work upon which most emphasis was placed and which seemed to be the most important from the standpoint of the plant breeder of the spring-wheat section was to determine if the stem-rust resistance of the durum variety could be obtained in vulgare segregates of this cross. As a consequence, a study of all the cytological phases of the problem was not attempted. A few of the cytological features that have more or less direct relationship to the problem are of sufficient interest to be recounted here.

The parents were studied to determine if they were typical of the species to which each belonged from the standpoint of chromosome numbers. The F_1 was not studied in all its phases, but the metaphase and anaphase of the first division showed that the cytological behavior was similar to that described by other workers for pentaploid hybrids. Several of the F_2 plants were very irregular in their chromosome behavior. Plant 567-6 had approximately 38 chromosomes. From two to six univalents were observed in side views of the metaphase of the first division. Many end-to-end bivalents were observed as well as many trisomes. Other irregular combinations were frequent. A polar view of a pollen mother cell in the metaphase of the first division is shown in Figure 1, A. The F_3 progeny of this plant varied in chromosome numbers, which is direct genetical proof that the F_2 parent did not have 21 pairs of chromosomes. Several of the plants of the F_3 line derived from this F_2 were as difficult to study as the parent. One of them, No. 9-32, is recorded as a 34-chromosome plant in Table 5, and a drawing of the polar view of the early anaphase of the first division is given in Figure 1, B. The univalents, as shown by side views of the metaphase of the first division, varied from none to four.

The F_2 plant, 571-14, the progeny of which was grown in the F_3 had 29 chromosomes. The pairing in this plant was very irregular. Several tetrasomes were found. A pollen mother cell in the metaphase of the first division with 15 chromosomes is shown in Figure 1, C. All the F_3 plants of this line had 14 pairs of chromosomes, which would indicate that the greater number of gametes formed by the F_2 parent had 14 chromosomes or that the gametes with 14 chromosomes were more effective in the fertilization process. One of the most interesting plants of this line (Table 8) is No. 34-14 which was almost completely sterile. The metaphase of the first division of this plant is shown in Figure 1, D. As far as can be determined, there seems nothing abnormal about the chromosomes here observed, so that an explanation of the high degree of sterility in plants of this type can not be based on chromosome number.

The interesting F_3 segregates from the standpoint of the main purpose of this study are those with 21 pairs of chromosomes and stem or leaf rust resistance. Plant No. 7-28 (Table 3) has 21 pairs of chromosomes and is the equal of the durum parent in resistance to stem rust. The metaphase of the first division, showing 21 chromosomes, is given in Figure 1, E. The F_3 plant, No. 13-1, also has 21 pairs of chromosomes and is as resistant to leaf rust as Velvet Don. A pollen mother cell showing the metaphase of the first division with

21 chromosomes is illustrated in Figure 1, F. Univalents are sometimes found in these plants, but no more frequently than in the vulgare parent. A photomicrograph of a pollen mother cell of plant No. 7-1 shows two lagging univalents. (Pl. 1, A.)

Plant 21-35 showed 21 chromosomes at the metaphase of the first division—18 bivalents and 3 univalents. An anaphase of this plant is shown in Figure 1, G. The three univalents appear in this figure just after they have split equationally. This illustrates the usual behavior of unpaired univalents in plants in which bivalents and univalents are found together. A photomicrograph of the same cell is shown in Plate 1, D. This plant was completely sterile.

Another recombination which was obtained from this cross was the 14-chromosome number of the durum parent and the vulgare susceptibility to stem rust. This type is illustrated by plant No. 56-18, the chromosomes of which are shown in Figure 1, H. Another plant, No. 32-14, shows this same combination. A photomicrograph of the metaphase of the first meiotic division of the latter plant is given in Plate 1, B.

An illustration of a polar view of the metaphase of the first division of another 14-chromosome plant is shown in Plate 1, C. In this, the chromosomes are separated more widely on the equatorial plate than was the case in Plate 1, B. Accurate counts can often be made in the early anaphase of the first division. (Pl. 1, E.) Counts of both groups should be made, however, before the total number in the cells can be determined. This is true especially in plants with unpaired univalents. The two parts of the cell are found often in adjacent sections of the slide. If the members of the bivalents are in the condition in which they can be counted, the univalents are still lagging and have not split equationally. They may be found then in one or the other of the parts of the cell. This frequently would give different numbers in the two sections. As an example, a 35-chromosome plant gave 19 and 16, 17 and 18, and 15 and 20 in counts of this kind. Such counts might be misleading from the standpoint of the behavior of unpaired univalents, since it might appear that they go to the poles by random in the first division.

A side view of the later anaphases of such plants frequently shows, however, the lagging unpaired univalents scattered around the equatorial plate in more or less advanced stages of equational splitting. This would indicate that they split at the first division.

DISCUSSION

The chromosome behavior in the cross, Velvet Don \times Quality, is similar to that reported by other workers for pentaploid hybrids. When bivalents and univalents are found in the same plant, there is evidence that the bivalents behave normally in both divisions, but that the univalents lag, come to the plate later in the first division, split equationally, and join the members of the bivalents at the poles.

The fact that there are fewer plants among the F_2 segregates with an intermediate number of chromosomes is explained by Sax (13) as due to the elimination of gametes with intermediate numbers of chromosomes, because they are less fertile and less viable than those with either 14 or 21 chromosomes. The absence of a complete set of chromosomes results in gametic sterility, and the absence of a com-

plete diploid set results in sterility in the zygote. Thompson and Cameron (15) suggest that the plants with intermediate chromosome numbers occur less frequently in F_2 and certain F_3 lines, not only because of gametic elimination, but also because of zygotic mortality. Kihara (7) divided the possible combinations into fertile and sterile groups on the basis of the number of bivalents and univalents which they possessed. Many of the intermediate types are given as fertile combinations. As evidence that the number of bivalents and univalents in the combination is not always a reliable index of sterility, the work of A. A. Sapehin (9) may be cited again. He obtained a 36-chromosome plant having 16 bivalents and 4 univalents which remained constant in morphological characters and chromosome number for six years. A combination with the same number of bivalents and univalents was given by Kihara as sterile.

Further evidence that combinations of certain numbers of bivalents and univalents do not always determine the degree of sterility has been obtained in the Velvet Don \times Quality cross reported in this paper. One of the F_3 segregates, No. 21-35 (Table 6), had 18 bivalents and 3 univalents but was completely sterile. This combination was reported by Kihara as fertile. In this cross, too, one F_3 segregate with 14 pairs of chromosomes was completely sterile, while three others were almost in the same condition. These plants have a well-balanced condition as far as chromosome numbers are concerned. It is not reasonable to suppose, however, that the chromosomes were all derived from the original durum parent, since, although the plants have durumlike morphological characters, they show complete or very high degree of sterility. They must, therefore, represent a combination of durum and vulgare chromosomes that is out of balance from the standpoint of genetic factors. This condition must have resulted because of an exchange of chromosomes or parts of chromosomes which are different in quality between the two parent species.

Sax (13) concludes that the 14 emmer chromosomes are essentially similar to the 14 vulgare chromosomes with which they pair, that the 7 extra vulgare chromosomes determine most of the typical characters of this group of wheat species, and that the desirable and typical characters of the emmer and vulgare wheats can seldom if ever be combined. The cytologic and genetic data obtained from the cross, Velvet Don \times Quality, show that recombinations between the typical characters of the durum and vulgare parents occur with relative frequency. Some association between chromosome numbers and morphological characters is apparent, but relatively homozygous fertile segregates in which are combined the valuable characters of both species occur in sufficient numbers to make feasible the use of such a cross in plant breeding. Several plants were found in the F_3 which combined vulgare characters and chromosome numbers with the stem-rust resistance of the durum parent. The leaf-rust resistance of Velvet Don was found in one segregate of the vulgare type and chromosome number. Several plants with 14 chromosomes and morphological characters of the durum had the stem rust susceptibility of the vulgare parent. Both stem and leaf rust resistance were combined in some of the 14-chromosome durumlike segregates, but in no case was resistance to these two diseases present in the same 21-chromosome vulgare plant. To find a plant of the latter type

resistant to leaf rust and another one of the same type resistant to stem rust is a step in advance, however, since a cross between these two probably would give the desired recombination without the difficulties encountered in a species cross.

The amount of seed setting in the F_1 of this cross, 5 per cent, makes it possible to get a large F_2 population with a reasonable amount of work. Sterility in the F_2 from whatever cause, eliminates a number of segregates, but some of the fertile ones should give the desired results. If apparently homozygous fertile segregates combining the valuable characters of the vulgare and durum parents are found in the relatively small numbers which can be used in a cytologic study, it is reasonable to expect that almost any combination can be obtained when large numbers are used, as is done in a comprehensive genetic study. Evidence of the same nature is given by Thompson (14) from results obtained in a combined genetic and cytologic study. He found that, while there is a rather definite association between chromosome number and other characters in each of the species, the correlation between stem-rust resistance and durum characters had been broken, although he found no vulgarelike plants as resistant as the durum parent. The important breeding results which have been obtained by Hayes et al. in the production of Marquillo wheat, by McFadden in the production of Hope wheat, by A. A. Sapehin in the production of vulgare strains of wheat resistant to the attacks of Hessian fly and to drought, and by Nina Meister (8) who reported the production of drought-resistant and winter-resistant strains of wheat from wheat-rye crosses, all point to the fact that species hybrids can be used as an important means of obtaining varieties of value from an economic standpoint.

The facts obtained in all the studies enumerated seem to be contradictory to the hypothesis stated by Sax. It is true that the cytologic and genetic information is still fragmentary and not entirely conclusive, and that much more work will have to be done before all the questions of sterility and of chromosome and genetic behavior can be answered in detail. Enough is known already, however, to assure the plant breeder that if sufficiently large numbers are used there is every reason to believe that any desired recombination of the characters of the parents of a species cross can be secured.

SUMMARY

The relationships between chromosome numbers, characters of keel, collar, stem cavity, glume shape, reaction to stem rust, and reaction to leaf rust were studied in segregates of a pentaploid hybrid, *Triticum durum*, variety Velvet Don, \times *T. vulgare*, variety Quality.

The F_1 had a total of 21 chromosomes in the metaphase of the first division, 14 bivalents and 7 univalents. The F_1 plants had the durum type of keel and collar, an intermediate condition for stem cavity and glume shape, intermediate condition for beards, and the pubescence and red seed color similar to the durum parent. They were susceptible to stem rust. They set seed to the extent of about 5 per cent.

The chromosome behavior in this cross was for the most part similar to what was been reported for such hybrids by other investigators. When bivalents and unpaired univalents are found in the same plant, the bivalents behave normally in both meiotic divisions. The unival-

ents lag, come to the plate late, divide equationally, and join the members of the bivalents at the poles.

Association between the chromosome number of each species and the typical characters of the same is apparent, but recombinations of the characters of both species are relatively frequent.

Relatively homozygous, fertile F_3 segregates with the vulgare chromosome number, keel, collar, stem cavity, and glume shape combined with the stem-rust resistance of the durum have been obtained. One fertile vulgarelike F_3 segregate had the leaf-rust resistance of the durum.

Durumlike plants with 14 chromosome pairs were found that were as susceptible to stem rust as the vulgare parent. All the plants of one F_3 line had 14 pairs of chromosomes and durumlike characters, but with an intermediate type of resistance to leaf rust, although they varied somewhat in this respect.

One F_3 plant with 14 pairs of chromosomes was completely sterile, and three others almost sterile, giving only a very few shriveled seeds. One F_3 plant with 39 chromosomes was completely sterile. Another with 34 chromosomes gave only a few shriveled seeds.

The relative frequency with which recombinations of the characters of the two species occurred is evidence that a cross of this kind can be used to advantage in plant breeding and is contrary to the conclusion that the desirable and typical characters of the emmer and vulgare wheats can seldom, if ever, be recombined.

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ANATOMY AND PHYSIOLOGY OF THE DIGESTIVE TRACT OF THE JAPANESE BEETLE¹

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INTRODUCTION

The Japanese beetle (*Popillia japonica* Newm.) is a phytophagous insect, known to feed upon more than 200 species of plants. The study that follows presents the results of an effort to describe the structure of the digestive tract of the beetle and its ability to digest certain classes of foods. The investigation was conducted at the Japanese beetle laboratory, at Riverton, N. J.

Specimens for histological work were fixed in Carl's fixative and stained in Delafield's haematoxylin and eosin, and those for work on acidity and enzyme analysis were used directly, without killing.

ANATOMY

The digestive tract of the Japanese beetle is approximately twice as long as the body. The diameter ranges from one-half to 1 mm., and is generally greater at the anterior end than at the posterior. The tract begins as a straight tube leading back through the head and thorax, and after entering the abdomen coils about itself, filling the major part of the cavity. The hind-gut lies on the dorsal side of the mass, the mid-gut being beneath. The appearance of the different layers of the tract depends upon the quantity of food passing through. When the tract is empty the fore-gut will shrink slightly, thus causing its walls to be thrown into folds, but this condition is only temporary; when food is taken in, the walls become flat and stretch somewhat. The esophageal valve becomes less prominent and stretches so as to form a large passageway between the fore-gut and mid-gut. The mid-gut also has a different appearance when empty. Its cells seem to become longer and very thin, and the epithelial wall is thrown into large folds which practically close the gut. These folds are always present when the gut is empty, but their presence can not be detected on the outer wall. The anterior part of the hind-gut does not lose its usual appearance, but the posterior part expands during heavy feeding and may almost eliminate the longitudinal folds so as to form a circular interior. In this insect, therefore, many of the folds in the epithelium seem to occur only when the gut is empty, and are formed by the contraction of the muscles. Where the folds are very deep, as shown in Figure 3, B, they are not entirely eliminated by the pressure of the food, and may be more or less permanent. The gross anatomy of the tract is fairly generalized, the tract consisting of fore-gut, mid-gut, and hind-gut.

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THE MOUTH PARTS

There are seven well-developed mouth parts in this beetle; the labium, hypopharynx, left and right maxillae, left and right mandibles, and labrum.

The labium (fig. 1, E) is a flat plate lying on the ventral side of the head, and serves to protect the movable mouth parts from beneath.

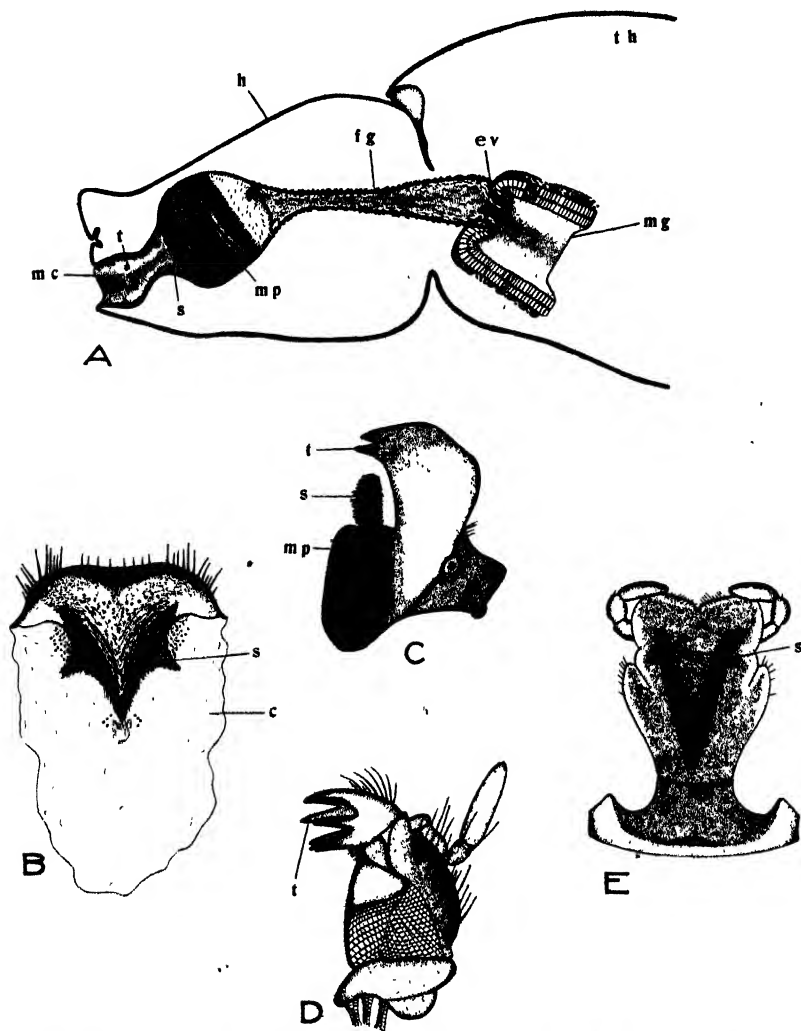


FIGURE 1.—A, longitudinal section through head and thorax of the Japanese beetle, showing right half of forepart of the tract, $\times 20$; B, ventral surface of labrum, $\times 60$; C, dorsal view of right mandible, $\times 30$; D, dorsal view of right maxilla, $\times 30$; E, dorsal surface of labium, $\times 35$; c, cuticula; fg, fore-gut; h, head; mc, mouth cavity; mg, mid-gut; mp, mandibular plate; ev, esophageal valve; s, spines; t, teeth; th, thorax

Its posterior region, called the submentum, broadens out to attach the labium to the main sclerite of the head. Anterior to this is a large plate called the mentum, which forms the major part of the labium. On either side there is a 3-segmented labial palpus.

Immediately above the labium there is a triangular plate called the hypopharynx. Its anterior end is attached to the labium by a thin fold of the body wall, and its entire surface is covered with stout spines. It is located in the center of the labium, and its pyramid-shaped body arises between the two maxillae.

On either side, and above the hypopharynx, there is a hard maxilla. The outer margin of this organ (fig. 1, D) is covered with stout spines, and the innermost part, the galea, is fitted with six long teeth. The teeth form a triangle, with three in the basal row, one on each side, and one at the apex.

Above each maxilla there is a hard mandible (fig. 1, A, C) with two small teeth on the inner apex. The base of each mandible is triangular, and the inner surface is provided with a D-shaped plate which fits into the anterior opening of the fore-gut so as to hold it open for the passage of food.

Above and in front of the mandibles there is a small, triangular plate, the labium (fig. 1, B) the base of which faces the front and is covered with spines. The apex is in the center of the mouth cavity directly above the hypopharynx.

The labrum is attached to the clypeus above, and is not movable in any direction. The mandibles and the maxillae swing laterally only, and with a forward-and-backward motion produce a rasping effect on the plant tissue. The labium is hinged at the posterior edge, and can be moved vertically. When the insect is feeding, the labial plate is pushed downward and serves to regulate the space required by the grinding mouth parts. Two chitinous plates, one at the base of each mandible, fit into the anterior opening of the fore-gut, one on either side, and serve to hold the gut open and to grind the passing food. The inner surfaces of these plates are ridged and fringed with a row of short spines or teeth. The inner surface of the mouth cavity is lined with a thick intima.

THE SALIVARY GLANDS

This insect apparently has no salivary glands, as none can be demonstrated among the tissues of the head and thorax, and a histological study of the entire system shows no evidence of a salivary duct. This conclusion is strengthened by the failure of physiological methods of investigation to demonstrate a salivary enzyme in the gut or its contents.

THE FORE-GUT

The fore-gut (fig. 1, A, fig. 2, A) is about 2 mm. long and lies almost entirely within the head of the insect. For 1 mm. back of the mouth it is threadlike; the posterior half widens out into a pear-shaped bulb. It is about one-third of a millimeter in width, and has a translucent, waxy appearance. As has been stated, the anterior end of the fore-gut is held open by two plates, the opening being about 0.5 mm. in diameter. The walls are muscular, and have a pumping action imparted by two bundles of muscle fibers which extend from either side of the dorsal wall of the tract to the dorsal wall of the head.

Between the fore-gut and mid-gut lies the esophageal valve, a ring of epithelial cells which form a constriction between the two regions. This valve is not elaborate, and, being very small, does not appear to have any great functional value. In fact, it scarcely

functions as a valve, for the contents of the mid-gut readily regurgitate into the fore-gut, in which is commonly found material which has been in the mid-gut.

THE MID-GUT

The mid-gut (fig. 2, A) constitutes the major portion of the digestive tract. It ranges from 2 to 2½ cm. in length and from one-half

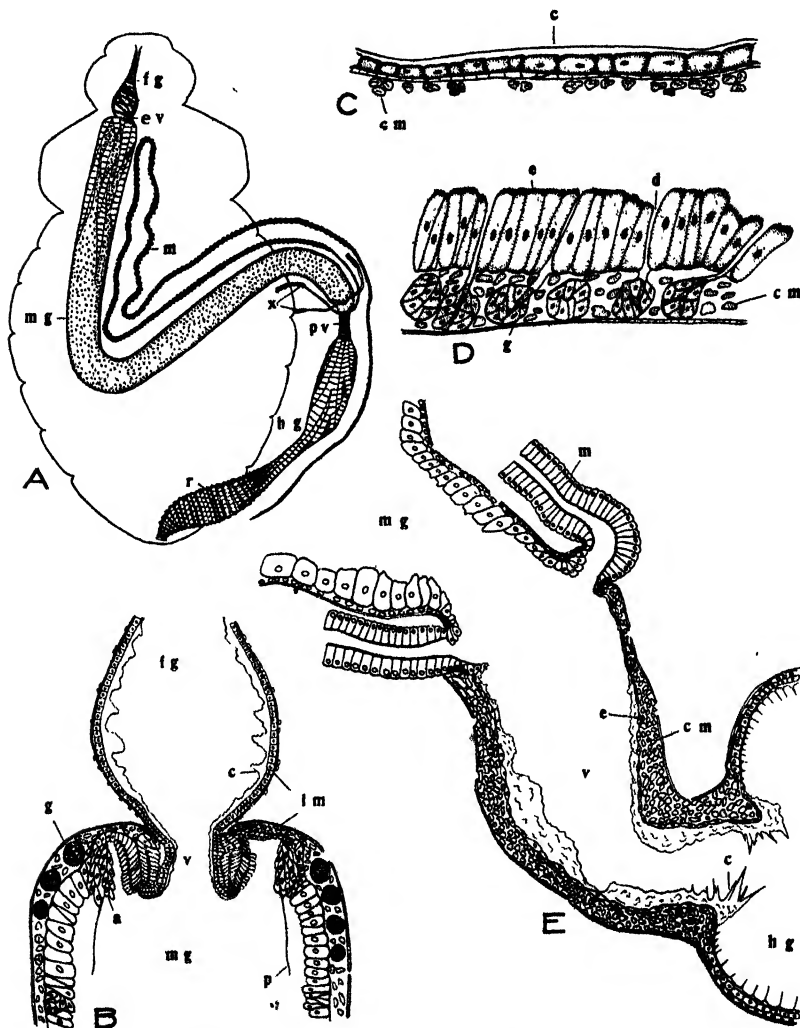


FIGURE 2.—A, Dorsal view of entire digestive tract, partially dissected from the body cavity, $\times 6$; B, longitudinal section through esophageal valve, $\times 75$; C, longitudinal section of wall of fore-gut, $\times 160$; D, longitudinal section of wall of mid-gut, $\times 160$; E, longitudinal section of pyloric valve, $\times 160$; a, collar of cells secreting peritrophic membrane; c, cuticula; cm, circular muscles; d, duct leading from nidus; e, epithelium; ev, esophageal valve; fg, fore-gut; g, nidus; hg, hind-gut; lm, longitudinal muscles; m, Malpighian tube; mg, mid-gut; p, peritrophic membrane; pv, pyloric valve; r, rectum; v, valve; x, Malpighian tubes, partially drawn

to 1 mm. in width. It is wider at the anterior end (premid-gut) and narrows gradually toward the posterior end (postmid-gut). This gut leads back from the head into the abdomen, where it is

coiled about itself to accomodate its length. It is only moderately muscular, and its surface is covered with minute, knoblike organs which are called nidi, and which form new cells for the epithelium of the mid-gut.

THE HIND-GUT

The beginning of the hind-gut is marked by the presence of four Malpighian tubes. These are long, lacelike tubes (fig. 2, A), two of which cling to the wall of the tract and run anteriorly to the esophageal valve, where they turn and run posteriorly to the colon. The other two are about half the length of the first and have a common opening into the gut.

Posterior to the Malpighian tubes is the pyloric valve, which is about 1 mm. long and has a very small diameter. It is extremely muscular and is a very efficient valve.

Beyond the valve the hind-gut widens, and has a very thick, opaque, yellow wall, which is usually wrinkled and pitted. It narrows near the middle, for a very short distance, and is relatively thin-walled and translucent. After widening again to form a large rectum, it terminates in the anus. The rectum, like the pyloric valve, is very muscular. Rectal glands are not developed in this insect, the walls being folded but not thickened in any way. The entire hind-gut is slightly more than 1 cm. in length.

HISTOLOGY

In the following study the parts of a hypothetical gut (that is, one which has lost none of the structures found in embryonic forms) are enumerated, and this hypothetical gut is compared with the gut as actually found in the Japanese beetle.

FORE-GUT

In any such hypothetical digestive tract as has here been postulated the fore-gut would have, if, beginning at its inner surface, the successive layers or essential parts be named in the order of their occurrence, an intima, an epithelium composed of ectodermal cells, a basement membrane, a layer of longitudinal muscles, and, completing the assemblage of parts, an outermost layer of circular muscles.

The fore-gut of the Japanese beetle (fig. 2, C) has not deviated to any great extent from this hypothetical form. The inner wall is covered with a rather thick intima, which is greatly folded when the gut is empty, but becomes thinner and flattened when the gut is distended with food. At the anterior end the intima is rather thick and forms teeth, or spines, which extend into the canal. Beyond this it is thin and wavelike, and continues to be such through the esophageal valve.

The epithelium is uniform from the mouth to the valve, and consists of pavement cells which are of about the same size throughout the gut. When the fore-gut is distended with food these cells stretch and have the appearance of merely a thin line. The basement membrane is not evident. If present it is extremely thin and clings very closely to the base of the epithelial cells in such a way as to become imperceptible.

The layer of longitudinal muscles is very thin, but well defined. It fits very closely against the epithelium, and is so continuous as to form an almost perfect sheath about the gut. The circular muscles are well defined, but are present in scattered strands only.

The fore-gut is formed by an invagination of the body wall, and is therefore ectodermal in origin. It is not highly specialized, and its major function seems to be that of transferring food from the mouth to the mid-gut.

ESOPHAGEAL VALVE

As the esophageal valve is a modified portion of the fore-gut the hypothetical histology is the same for both. In the actual valve the intima is the same as that of the fore-gut, and extends to the tip of the epithelial layer of the valve. The epithelial layer also is similar to the anterior part of the fore-gut, and is not specialized. The muscular layers are not present. The valve is held in position by a ring of epithelial cells of the mid-gut, which form a collar about it. There are no sphincter muscles in the valve, which is relatively inefficient.

MID-GUT

The mid-gut of the hypothetical digestive tract would consist of an epithelium composed of endodermal cells, a basement membrane, a layer of circular muscles, and one of longitudinal muscles.

The anterior end of the actual mid-gut (fig. 2, B) has a ring of modified epithelial cells which form a collar about the esophageal valve, and secretes the thin peritrophic membrane. This membrane has no cellular structure and serves merely to protect the delicate epithelium from rough particles of food. It is probable that the entire membrane is secreted by the ring of cells, for it clings tightly to the food within. It is secreted as the food passes through the esophageal valve and is drawn by the passage of the food through the gut. Whether the epithelium along the gut aids in the secretion of the membrane has not been determined.

The epithelium of the mid-gut is composed of columnar cells, and its anterior end forms a circular fold which surrounds the esophageal valve. Beyond this point the cells in the epithelium appear to vary but little throughout the length of the gut. Those of the postmid-gut are slightly flattened, but the difference between them and the other cells is not great. They are composed of rather dense protoplasm which has a granular appearance. The nuclei are small and are filled with granules of chromatin.

The type of secretion could not be determined from the sections obtained, but it is evidently holocrine, or brought about by the destruction of cells. In this type of secretion new cells must be formed to take the place of those breaking down. This formation is accomplished by the division of cells within round, cellular aggregations called *nidi*, most of which have dropped below the epithelium. Some lie among the strands of circular muscles, while others have passed through the muscular layers and appear on the outer surface. In dropping below the epithelium they push the basement membrane before them, and thus utilize it as an outer membrane and as a passageway for the new cells up to the epithelial layer. Within each *nidus* is a group of small cells closely packed together. They are all of equal size, and do not form the usual budlike *nidus* in which the

size of the cells increases from the center outward. The cells pass out through the duct formed by the basement membrane and take their place in the epithelium above. A nidus may occasionally be found among the cells of the epithelial layer, but this situation is not so common as the type already mentioned. Beneath the epithelial cells there is a well-defined basement membrane, which also forms the walls and ducts of the nidi, as already mentioned.

The circular muscles are fairly well developed. They are present throughout the entire length of the mid-gut and serve to force the food through the tract. The longitudinal muscles are fairly well preserved at each end of the mid-gut, but are either discontinuous or absent through the median section. They are very thin, and are probably not important factors in the functioning of the gut.

MALPIGHIAN TUBES

The posterior end of the mid-gut tapers into a conelike process which enters the pyloric valve. Four Malpighian tubes (fig. 3, A) arise at the point of entrance, two of which open on either side of the gut, while the other two have a common opening slightly above them. The tubes are simple and straight as they leave the gut, but slightly above this point they have a fine, lacelike appearance, and each upon magnification appears as a distorted tube with many spherical lobes protruding from its surface.

The inner surfaces of the Malpighian tubes are lined with a very thin intima, since they are embryologically a part of the hind-gut. The remainder of the tube is made up of a layer of columnar cells with their nuclei at the outer margin, and the lobelike projections mentioned above are caused by the irregular contour of this layer, which is not thickened at the lobes, but curves in and out to form them. The canal within the tube is fairly large, and in living beetles the contents may readily be seen flowing down the tube. The contents could not be detected in the stained sections, but in living material they have the appearance of white granules in a colorless liquid matrix.

PYLORIC VALVE

The hypothetical pyloric valve and, since its histology is the same, the hypothetical hind-gut consist, from the inner surface outward, of an intima, an epithelium of ectodermal cells, a basement membrane, and three layers consisting, respectively, of inner circular muscles, longitudinal muscles, and outer circular muscles.

In the pyloric valve the intima is very well developed. It is not present in a solid sheet but appears in very thin, wavelike folds, which become thickened at the posterior end of the valve, where they form large teeth or spines extending into the gut. (Fig. 2, E.)

Beneath the intima is a layer of very thin ectodermal cells, scarcely visible in some sections. These cells are elongate, thick around the nucleus at the center, but thinning rapidly toward the margins. The basement membrane is not distinct from the epithelium above it.

The inner, circular muscles are very well developed, forming the major portion of the wall of the valve. They are packed closely together, sometimes three to five strands deep. These muscles probably have the power of closing the valve entirely. There is a fairly

well-developed layer of longitudinal muscles, one strand in thickness, which is almost continuous about the valve. The outer circular muscles are lacking.

HIND-GUT

The histology of the hypothetical hind-gut is the same as that of the hypothetical pyloric valve. The intima varies in this gut, and appears

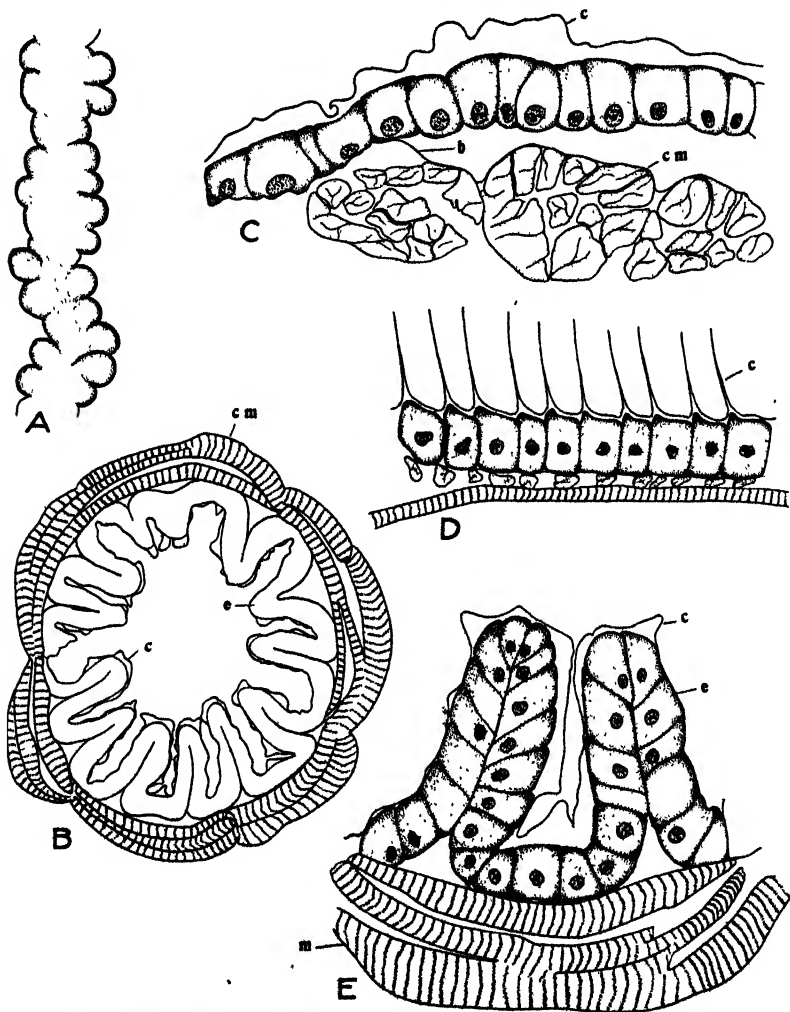


FIGURE 3.—A, Malpighian tube, external view, $\times 90$; B, cross section through posterior part of hind-gut, $\times 135$; C, longitudinal section of wall of posterior part of hind-gut, $\times 375$; D, longitudinal section of wall of anterior part of hind-gut, $\times 630$; E, cross section of two of the folds in posterior part of hind-gut, $\times 585$; b, basement membrane; c, cuticula; cm, circular muscles; e, epithelium; m, Malpighian tube

in two different forms. In the anterior portion it is drawn out into long spines which extend into the canal. (Fig. 3, D.) These spines are needlelike and are approximately of the same number as the cells beneath. If dissected immediately after emergence and before the

beetles have had a chance to feed, this gut will appear to be very small and shriveled; in fact, before feeding, it generally has the same diameter as the pyloric valve.

When the insect begins to feed, the finely ground particles of food pass down the tract and, on entering the hind-gut, become lodged among the spines which protrude from the walls. After the insect has fed sufficiently the inner wall of this region becomes completely coated with a layer of food, until finally only a small passageway may be observed down through the center of the gut. Whether this material remains stationary throughout the life of the beetle, or whether it moves posteriorly, is not clear. It is tightly packed within the gut and, unlike ordinary loose particles of food, is readily stained with eosin. In washing the gut in preparation for physiological studies this layer was not cleared away because any attempt to wash the gut clean would have disrupted the epithelium beneath. Toward the mid section of the hind-gut the tract becomes narrow, and the chitinous lining changes from sharp spines to an irregular layer (fig. 3, c), which is found throughout the remainder of the gut, and does not hold the food particles stationary.

The epithelium lies in a smooth cylindrical layer about the gut in the forepart of it, but gradually changes in the constricted portion of the mid section to a longitudinally folded layer. (Fig. 3, B, E.) The cells of the epithelium are large and of approximately equal dimensions. The protoplasm is less dense than in any of the other cells of the tract, and the nuclei are large and granular. There are no rectal glands, and there is no noticeable thickening of epithelial cells anywhere in the hind-gut. The basement membrane is present, and clings closely to the base of the epithelium.

The inner circular muscles are well developed throughout the gut, but those of the forepart are much reduced in size. (Fig. 3, D.) In the posterior half of the gut the inner circular muscles only are present and appear as enormously developed fibers. (Fig. 3, B, C, E.) Longitudinal muscles are present in the forepart of the gut only, and thin out and disappear in the mid region. (Fig. 2, A.) There are no outer circular muscles. The forepart of the hind-gut, therefore, is inclosed by two moderately developed layers of muscles, while the hindpart has but one layer, which is greatly developed.

HYDROGEN-ION CONCENTRATION

Determinations of hydrogen-ion concentration were made with a potentiometer and its accessories, equipped with a quinhydrone electrode. The writer has somewhat modified the usual arrangement of such apparatus, for the sake of testing small drops of liquid. A cabinet was constructed for the apparatus having a flat top on which was mounted a small platinum plate insulated by a block of sealing wax. (Fig. 4.) A wire from the galvanometer was soldered to this plate which served as one of the electrodes. The drop of liquid to be tested is placed upon this plate and the circuit is made by dipping in one end of the agar bridge from the potassium acid phthalate cell the drop, as shown in the figure. The agar bridge is a glass tubing containing agar saturated with potassium chloride. The potassium acid phthalate is a 0.05 m. solution. A small quantity of quinhydrone is mixed with the potassium acid phthalate and in the drop of

liquid to be tested. In this manner the hydrogen-ion concentration of a drop so small as to be only from 1 to 2 mm. in diameter could be determined. The electrodes of the outfit were washed each time with neutral water, the washing being repeated until the pH value was exactly 7, that is, neutral. With this precaution no reading could have been influenced by the one preceding.

A different beetle was dissected for each reading to prevent any post-mortem changes in the hydrogen-ion concentration of the contents of the tract. In the case of the fore-gut and the premid-gut the contents were secured by means of a very fine capillary pipette inserted into the wall of the canal, and were immediately placed upon the platinum plate and tested, the pipette being washed in

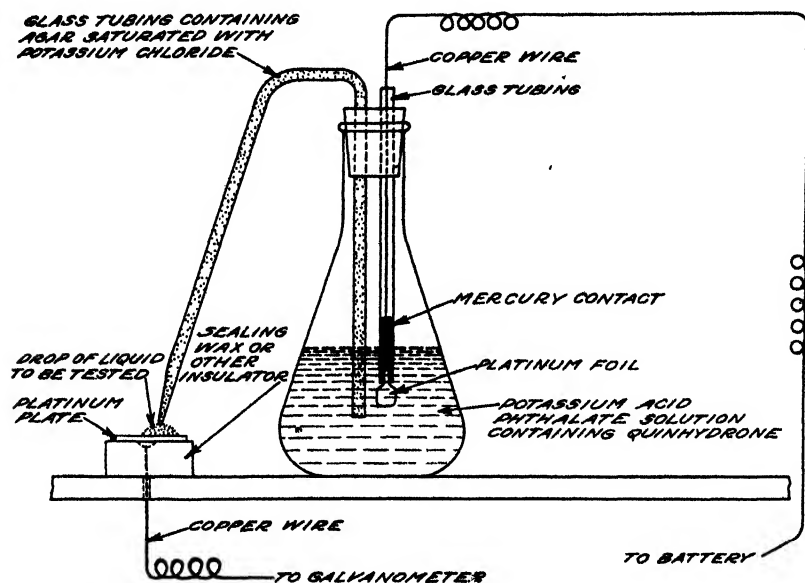


FIGURE 4.—Micro-quinhydrone electrode

neutral water before each use of it. The contents of the postmid-gut and the hind-gut were removed after the tract was cut open, a procedure necessary for the reason that the contents of the postmid-gut are in a semisolid condition. A small drop of neutral water was added to the feces, as otherwise they were much too solid for testing. Enough moisture was present without dilution in the postmid-gut and the hind-gut.

THE FORE-GUT

The fore-gut is about 2 mm. long and contains a liquid which is transparent in the esophagus and reddish brown in the crop. The transparent liquid is composed of plant juices and secretions of the fore-gut; it is not saliva, for there are no salivary glands in the beetle. Readings were taken on the contents of the fore-gut of each of five different beetles, with determinations of pH values as follows: 7.28, 7.28, 7.20, 7.37, 7.28; average, 7.28.

THE PREMID-GUT

The mid-gut is about 2.5 cm. long. The gastric caeca are not individual organs in themselves but are specialized cells in the walls of the mid-gut. Because of its length and the possibility of fluctuations in the hydrogen-ion concentration of its contents, the mid-gut was divided into a premid and a postmid portion, 0.5 and 2 cm. long, respectively. The material contained in the premid portion is a thin liquid of a dark reddish-brown color. Readings made on seven different beetles gave pH values of 7.37, 7.37, 7.23, 7.28, 7.46, 7.37, and 7.53; average, 7.37.

THE POSTMID-GUT

The postmid-gut is about 2 cm. long, and contains a very viscous, or partially solidified, mixture. This material is usually green in color when the beetle has recently been feeding. Readings made on nine different beetles gave pH values of 7.20, 6.94, 7.28, 7.70, 8.12, 8.21, 7.53, 6.77, and 7.95; average, 7.52.

THE HIND-GUT

The hind-gut, including the colon and rectum, is about 1 cm. long, and contains the undigested portion of the food, which is greenish yellow near the pyloric valve and changes to dark brown at the colon. Ten readings were made, indicating pH values of 7.70, 7.70, 7.53, 7.45, 7.87, 7.63, 7.32, 7.53, 7.87, and 7.37; average, 7.60.

THE FECES

Feces in the rectum are alkaline, the pH value being about 7.3 or 7.4. On exposure to the air, however, they quickly turn acid, so any feces defecated by the beetles normally would have an acid reaction. Feces taken by dissection from the rectum were found directly afterward to be alkaline, the pH being 7.2, but a few minutes later it had dropped to 7.0. After 10 minutes it may drop to 6.0 or 6.5. The hydrogen-ion concentration would therefore depend upon the time intervening before the test.

DIGESTIVE ENZYMES

The alimentary tracts of approximately 125 Japanese beetles were used in the work of ascertaining the presence of enzymes in them. The legs and wings were removed from each beetle, and the body wall was cut around the entire lateral line. The body was pinned, ventral side down, in a paraffin dish, after which the dorsal wall was removed by pulling upward with a needle and gently cutting away the tissue clinging to the underside. The entire alimentary canal was then removed by cutting just behind the mouth parts and at the posterior end of the rectum. It was pinned in a paraffin dish, and was slit from one end to the other with a microscalpel. The gut was then washed clean with a pipette of distilled water and transferred to a paraffin dish of fresh water. Here it was divided into four parts, the fore-gut, the premid-gut, the postmid-gut, and the hind-gut. Each of the four sections of the tract was then placed in its respective vial of neutral glycerin. After a sufficient number of beetles had been dissected, the contents of each vial were poured into a mortar and

ground into a homogenous fluid. The grinding served to crush all of the cells of the tissue and to liberate such enzymes as were present. A drop of brom thymol blue was then added to the mixture, so that changes from alkalinity to acidity could be observed and neutralized by the addition of small quantities of sodium acid carbonate. The material was then ready for use.

The tissues were washed free from the contents in order to determine just where the enzymes were located. If the contents were allowed to remain in them, it would be impossible to determine whether a specific enzyme had been secreted in the gut being tested or in the one preceding. The contents of each section of the gut include not only the enzymes secreted by that section, but all that have been received by it from the previous sections, and specific information can be obtained only when the tissues are washed.

On the other hand, the presence of any enzyme in a tissue does not necessarily mean that it is excreted into the tract at that point. Therefore, to determine whether a specific enzyme is excreted at its point of appearance in the tissue of the gut, tests must be made on the contents of each part. The contents of each of these parts of about 15 beetles were accordingly washed out into glycerin and placed in vials corresponding to the suspensions of ground tissue. After the addition of a drop of brom thymol blue and adjustment to a slight alkalinity, the solutions were ready for use.

Tests were made for the presence of seven different enzymes. In testing for the presence of any one of them, the substance known to be broken down by that specific enzyme was placed in a microtube and several drops of the suspension of tissue or contents was added. The remainder of the tube was filled with toluene to prevent the action of microorganisms. The tube was then set aside for from two to four days, when a test was made for the end product of digestion. For each tube used for a test a control tube was made up in the same way, except that the suspensions of tissue were first boiled to destroy the enzymes.

AMYLASE

By the process of hydrolysis starch changes to maltose in the presence of amylase.

Eleven different samples were tested for this enzyme—the extract and the contents of the fore-gut, the premid-gut, the postmid-gut, and the hind-gut, the feces, the entire tract and its contents, ground in glycerin, and the heads of 12 beetles, ground in glycerin.

To perform the test, 1 c. c. of a 0.5 per cent starch solution (boiled) was placed in a microtube, and 2 drops of glycerin extract of the sample to be tested were added. The tube was filled to the top with toluene and incubated for 72 hours at room temperature. After incubation, 2 drops of a potassium-iodide solution of iodine were added to each tube. In all cases the contents of the tubes turned a dark blue, showing the presence of starch. In 100 hours no digestion was observed. After four or five days, however, some of the tubes showed evidence of a slight digestion, probably caused by bacteria of the posterior region of the tract.

Each sample was then submitted to the picric-acid test for reducing sugars. Four drops of incubated solution, 1 drop of 10 per cent solution of sodium hydroxide, and 2 drops of a saturated aqueous

solution of picric acid were placed in a microtube in the order named. The tube was then heated very gently over a hot plate. When a reducing sugar is present, the yellow acid is reduced to the reddish-brown picramic acid. This test must be checked before using, as a slight excess of sodium hydroxide may cause the formation of picramic acid without the presence of reducing sugar. Through an interval of 100 hours the test failed to indicate the presence of sugar in any of the tubes.

Fehling's test and Fluckiger's test, for sugars, were each applied to the 11 samples named. In these tests a red precipitate of copper appears when maltose is present. No such precipitate was formed in any case.

The entire process, as just outlined, was repeated several times, with variations in the acidity or alkalinity, the percentage of starch, and the electrolytic content of the solution. Similar results were obtained.

As a result of the tests applied it was concluded that the enzyme amylase was not demonstrated in this insect. The feces and contents of the hind-gut appear to contain bacteria that have the property of digesting starch after long periods of time. Starch is apparently not converted to maltose in the alimentary tract of the Japanese beetle.

MALTASE

Maltose is hydrolyzed to glucose in the presence of maltase.

One cubic centimeter of a 3 per cent solution of maltose and 2 drops of glycerin extract of the sample to be tested were placed in a microtube; the tube was filled to the top with toluene, and incubated for 96 hours at room temperature. Barfoed's test for monosaccharides was then applied. In this test a small quantity of acetic acid is added to a solution of copper acetate, which is used to restrain the reaction of the disaccharide, because, if the solution is too alkaline, the disaccharide may be hydrolyzed to glucose without the presence of the enzyme. To detect the possible presence of glucose the reagent should therefore be checked with a solution of maltose before using. If glucose is present, a reddish-brown precipitate will appear on heating. The solution should not be boiled more than one-half minute, as prolonged boiling may hydrolyze the disaccharide. As a result of the test, only the tissue of the hind-gut gave a negative reaction. The microtubes containing the tissue of the fore-gut, the premid-gut and the postmid-gut, the feces, and the contents of the fore-gut, premid-gut, postmid-gut, and hind-gut were found to contain glucose, these results leading to the conclusion that maltase is secreted by all parts of the digestive tract except the hind-gut.

INVERTASE

In the presence of invertase the disaccharide sucrose is converted into two monosaccharides, fructose and glucose.

One cubic centimeter of a 15 per cent solution of sucrose and 2 drops of glycerin extract were placed in a microtube, and the tube was filled to the top with toluene and incubated 72 hours at room temperature.

The picric acid test for reducing sugars was then used. Three drops of the incubated solution, 1 drop of 10 per cent sodium hydroxide solution, and 2 drops of saturated aqueous solution of picric acid

were heated gently for 1 minute. In the presence of a reducing sugar the yellow picric acid is reduced to brown picramic acid.

The tissues of the four portions of the intestinal tract, the contents of the four, and the feces were tested, as was done in testing for maltase, and with precisely similar results; invertase was found in all the substances tested except the tissue of the hind-gut.

By way of confirmation, Fluckiger's test for reducing sugars and the test by reduction of methylene blue were applied to the same substances as before, and with similar results throughout. The conclusions of all these tests parallel those of the tests for maltase—invertase is secreted by all parts of the digestive tract except the hind-gut.

LACTASE

Lactose is hydrolyzed to glucose and galactose in the presence of lactase.

One cubic centimeter of a 1 per cent solution of lactose and 2 drops of glycerin extract of the sample to be tested were placed in a micro-tube, and the tube was filled to the top with toluene and incubated for 72 hours at room temperature. Barfoed's test for monosaccharides, already described, was applied to the several substances heretofore mentioned, with the result that no copper precipitate was formed. In the face of this negative result it can only be said that lactase was not demonstrated in this insect.

LIPASE

Complex fats are broken down into fatty acids and glycerin in the presence of lipase.

Two drops of brom thymol blue were added to 25 c. c. of a 10 per cent solution of milk (condensed milk), and either powdered sodium bicarbonate or a 1 per cent solution of potassium hydroxide was added until the solution became a light blue in color.

One cubic centimeter of the blue-milk solution and 2 drops of glycerin extract of the sample to be tested were placed in a microtube, and the tube was filled to the top with toluene and incubated 24 to 48 hours at room temperature. Since milk is an emulsion of a fat its digestion will produce fatty acids which color the blue milk yellow. This experiment was repeated, an olive-oil emulsion being used in place of the milk. The results were the same, although the reaction was slower. In both cases the change of color marking the reaction was observed in the case of the premid-gut and the postmid-gut, but the tests applied to the fore-gut and hind-gut gave only negative results. The feces and the contents of the four tracts gave positive results. These observations lead to the conclusion that lipase is secreted by the mid-gut only. Its appearance in the contents of the fore-gut is due to regurgitation from the mid-gut.

TRYPTASE

Complex proteins are broken down into proteoses, peptones, peptides, and amino acids in the presence of tryptase in an alkaline solution.

About 1 gm. of blood fibrin was placed in a solution of aniline blue and allowed to stain for several hours. It was removed and washed in alkaline water until no more of the blue stain could be washed from

it. The fibrin was then cut into pieces about 3 mm. square. About 0.5 c. c. of glycerin extract of the sample to be tested, together with 0.5 c. c. of water and a small bit of blue fibrin, was placed in a microtube, the pH was adjusted to 7.5, and the tube was filled to the top with toluene and incubated 96 hours at room temperature. The same substances were tested as with lipase and with correspondingly similar results. The blue color of the solution, imparted by the aniline blue released by the digestion of the fibrin, indicated the presence of tryptase in the premid-gut, the postmid-gut, the feces, and the contents of the four parts of the digestive tract. This evidence leads readily to the conclusion that tryptase is secreted by the mid-gut only. Its appearance in the contents of the fore-gut is due to regurgitation from the mid-gut.

PEPTASE

Complex proteins are broken down into proteoses, peptones, and peptides in an acid solution in the presence of peptase.

One gm. of blood fibrin was stained in a solution of amaranth red for several hours, then removed and washed in acidified water. When the red color no longer came out, it was cut into pieces about 3 mm. square.

About 0.5 c. c. of glycerin extract of the sample to be tested, together with 0.5 c. c. of water and a bit of the red fibrin, was placed in a microtube, the pH adjusted to 5, the tube filled to the top with toluene, and the contents incubated 96 hours at room temperature. In this test the red color is liberated and stains the solution if the fibrin is digested, which can be done only by a peptase. The same tissues and other substances were tested as in the case of the other enzymes considered, but without result; the presence of peptase in the digestive tract of the Japanese beetle was not demonstrated.

For convenience the results of the enzyme tests are summarized in Table 1.

TABLE 1.—*Summary of analyses for presence of enzymes in the digestive tract of the Japanese beetle*

[+=present; —=absent]

Enzyme sought	Substance analyzed	Results for sources of substances analyzed				
		Fore-gut (pH 7.28)	Premid- gut (pH 7.37)	Postmid- gut (pH 7.52)	Hind- gut (pH 7.90)	Feces (pH 7.25)
Amylase.....	Tissue.....	—	—	—	—	—
	Contents.....	—	—	—	—	—
Maltase.....	Tissue.....	+	+	+	—	—
	Contents.....	+	+	+	+	+
Invertase.....	Tissue.....	+	+	+	—	—
	Contents.....	+	+	+	+	+
Lactase.....	Tissue.....	—	—	—	—	—
	Contents.....	—	—	—	—	—
Lipase.....	Tissue.....	—	+	+	—	—
	Contents.....	+	+	+	+	+
Peptase.....	Tissue.....	—	—	—	—	—
	Contents.....	—	—	—	—	—
Tryptase.....	Tissue.....	—	+	+	—	—
	Contents.....	+	+	+	+	+

SUMMARY AND CONCLUSIONS

In the investigation reported in this paper a careful study was made of the anatomy and histology of the alimentary tract of the Japanese beetle, the hydrogen-ion concentration was determined, and the digestive enzymes were ascertained.

It was concluded that in the alimentary canal of the Japanese beetle there are no regions where the contents are acid. The contents of the canal are very weakly alkaline, being almost neutral in the fore-gut and increasing in alkalinity as they approach the colon. The hydrogen-ion concentration may vary slightly with the food eaten, but not to any great extent. The greatest variation is found in the postmid-gut, but as fats and proteins are digested here this variation may be due to different stages in digestion. The contents of the rectum are alkaline, but on exposure to the air in the form of feces they soon become acid.

No starch digestion could be demonstrated in the digestive system of the Japanese beetle. Maltose and sucrose are broken down into monosaccharides by the relatively strong enzymes maltase and invertase, respectively, which are secreted in the fore-gut, the premid-gut, and the postmid-gut. Fats are broken down into fatty acids by a single enzyme, lipase, which is secreted by the premid-gut and the postmid-gut. Proteins are broken down into proteoses, peptones, peptides, and amino acids by the enzyme tryptase, which is secreted by both parts of the mid-gut. Peptase was not found in the digestive tract. Secretion is carried on exclusively by the cells in the epithelial layer of the digestive tract of the Japanese beetle, as there are no specialized secretory glands in any part of the tract.

THE FEEDING RATE OF THE AUSTRALIAN LADY BEETLE, *RODOLIA CARDINALIS*¹

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INTRODUCTION

Although entomological literature contains abundant testimony to the efficiency of the Australian lady beetle (or *Vedalia* beetle), *Rodolia cardinalis* (Muls.), as a predator of the cottony-cushion scale, *Icerya purchasi* Mask., there are few quantitative data on the feeding of this beetle. An article from the Department of Agriculture and Commerce of Japan² gives the average consumption of the male and of the female for short periods, but the size of the scales and the factors which may condition the rate of feeding are not considered. The present paper presents the results of experiments designed to measure the effect of such factors on the adult beetles. These investigations are a part of a larger project which includes detailed studies of the relations between a predator and its host. The work was conducted at New Orleans, La., in the laboratory of the Division of Tropical, Subtropical, and Ornamental Plant Insects of the Bureau of Entomology.

METHODS

The beetles were confined in 18-mesh screen-wire cylinders, approximately 2¼ inches in diameter by 3½ inches high, with muslin tops and bottoms. They were fed on *Icerya purchasi* Mask. taken from infested *Pittosporum* bushes. When fresh leaves were used each day practically none of the scales left the screen cylinders. In a study of the movement of the scales it was found that when infested leaves were put in the cages, only 1 scale out of 364 was absent from the screen cylinders after 24 hours. On the basis of average weights (discussed later) this was a loss of less than one-half of 1 per cent. It was therefore possible to use a coarse-mesh screen, assuming that the beetles were actually subjected to the temperatures recorded by an insectary thermograph, which was checked daily against maximum and minimum thermometers.

In the first series of experiments observations were made daily. The records were taken between 8 a. m. and 8.30 a. m., a period when the temperature was near the minimum and when the beetles were eating less than at any time during the following 12 hours. In other series of experiments observations were made every four hours. In each case a record was made of the number of scales put into the cage and the number that remained at the time of the next observation, the difference giving the number eaten during that interval. Since there was a large difference in weight between scales of different ages, they were divided into three classes which were readily

¹ Received for publication Mar. 12, 1930; issued August, 1930.

² KUWANA, S. I. RESEARCHES ON THE COCCINELLID *VEDALIA* AND THE SCALE INSECT *ICERYA*. Japan. Dept. Agr. and Com., Injurious Insects and Pests Bul. 3, 107 p., illus. 1917.

distinguishable. "Small" scales were those of the first instar, "medium" scales included those of the second and third instars and a few of the fourth instar which differed little in size or appearance from the preceding instars, and "large" scales included the rest of the fourth instar and the adults. In order to eliminate one source of variation the large class was always removed from the leaves. Unfortunately an analytical balance was not available until these experiments were nearly completed. Weighings were then made of scales in the first two classes, and the numbers of scales eaten were converted to numbers of milligrams on the basis of average weights. The small scales were weighed in groups of 20, 500 scales giving a mean weight for this group of 1.936 ± 0.039 mgm.; the medium scales were weighed in groups of 4, 200 scales giving a group mean of 3.032 ± 0.093 mgm. The number of small scales, therefore, was multiplied by 0.0968, and the number of medium scales by 0.758 to give the milligrams of food consumed. This method introduces a source of variation, since there still remains a large difference in size of scales within the same class. However, no better method of recording the data was developed. It is not practicable to weigh the leaves at the beginning and end of the period, making determination and allowance for evaporation. Aside from the deposition of dust, eggs, and excrement on the leaves, the weight of the leaves is so great in proportion to the weight of the scales that variations in the evaporation rate would introduce enormous errors in calculating by weight differences the quantity of food eaten. It is believed that errors resulting from the method used have tended to average out because of the following considerations: *Icerya purchasi* produces continuously throughout the year and, consequently, in infestations of any age the scales are in all stages. Scales for all the daily observations and for most of the 4-hour ones were taken from two caged Pittosporum bushes which had been infested for over a year. During the course of the experiments no preponderance of any stage or stages was noted. Further evidence of the averaging out of the errors is found in the curves shown in the illustrations. Where each mean is the average of a considerable number of cases, the points fall rather closely into a smooth curve, a condition which would not be likely to exist unless there had been this averaging out of the errors introduced by the variation in size of the scales.

THE DAILY RECORDS

The records extended from September 3, 1925, to September 6, 1926, covering 11 broods of beetles, with a total of 2,152 observations, divided as follows: On pairs, 1,546; on single producing females, 324; on virgin females, 171; on virgin males, 111.

The 1,546 observations mentioned above were made on 75 pairs, the number of pairs to a brood ranging from 3 to 13. With the exception of a few records made on beetles kept under constant temperature, no experiments were conducted especially to obtain results from single females, but data were obtained in such cases, as the male died before the female.

It appeared possible that, in addition to temperature, several factors might affect the feeding rate of the beetles. The rate might vary with the age of the female. In some individuals the vital activities might proceed at a faster tempo than in others, in which case

under similar conditions there should be a difference in the average life span. There might be seasonal changes, as well as a progressive change with rearing in captivity and inbreeding. Accordingly correlations were made between the quantity of food consumed, in milligrams, and temperature, date (seasonal change), age, life span, and brood number (inbreeding), multiple curvilinear correlation methods being used.³ After two residuals has been calculated, the index of multiple correlation for the pair records, which include the majority of the cases, was found to be 0.81. For the single females only one residual was calculated with $R=0.69$.

The effect of temperature on the rate of feeding is shown in Figure 1. The rate increased more rapidly with higher temperatures, while below 10° C. the feeding, if not completely stopped, was negligible, although the beetles did not go into the dormant condition. In discussing the mechanism of such an effect it should be considered that food, in the case of the adult beetles serves ultimately as a source of

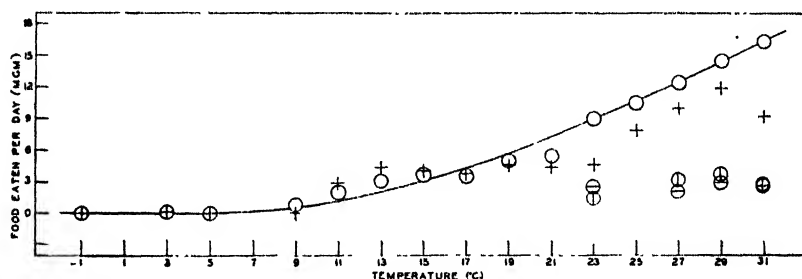


FIGURE 1.—Effect of temperature on quantity of food eaten by *Rodolia cardinalis*. Values for the pairs are represented by circles, values for single producing females by crosses, values for virgin males and for virgin females by circles with horizontal and vertical diameters, respectively. The smoothed curve has been fitted to the pair values. The graph has been fitted free-hand, but between 10° and 30° C. $y = -0.0207 + 0.9105x - 3.9480 \log x$ is an empirical equation giving a close approximation to the curve values

energy for (1) muscular movement, (2) replacement of tissue, and (3) production of eggs. The feeding rate of the beetles, therefore, might be conditioned by the foregoing processes, in which case it would be a resultant of several variables; or it might be directly a function of temperature, and itself the factor governing the rate of movement, egg production, and tissue repair; or the different activities might be independently affected by thermal change.

It is seen that the females ate the larger part of the food consumed by the pairs (the sex ratio was nearly 50 per cent, records on rearing 990 individuals showing 52.04 per cent females), but in contrast to the producing females, the virgin females showed about the same rate as the males. This fact indicates that feeding was dependent upon the rate of oviposition, since the virgin females produced only an occasional egg. If the feeding rate were directly a function of temperature, and the factor conditioning oviposition, no such difference should exist between producing and virgin females. The possibility of both being independently affected is also ruled out. Accordingly there should be a correlation between egg production and food consumption. (This should be true if either activity depended on the other,

³ EZEKIEL, M. A METHOD FOR HANDLING CURVILINEAR CORRELATION FOR ANY NUMBER OF VARIABLES. Jour. Amer. Statist. Assoc. 19: 444. 1924.

not if they were independently influenced by temperature.) In investigating this point the milligrams of food eaten and the number of eggs deposited were totaled for each individual, and correlations made on the basis of daily averages, a method necessary because of periodicities in the oviposition rate. Letting subscript 1 denote food, 2 eggs, 3 temperature, 4 life span, $r_{12.34} = 0.51$, $P = 0.01$.⁴ The effects of temperature and life span have been eliminated statistically, so that there was measured only the association of variations in the individual average feeding, independent of these influences, with variations in average oviposition. The more productive individuals were more effective not only because of their increased progeny, but also because they destroyed more scales during their lifetime.

In the case of vertebrates it is known that the hunger sensations arise from contractions in the empty stomach.⁵ While there is no direct evidence as to the existence of similar reactions in insects, the results presented above do indicate that an internal stimulus, dependent on utilization of food consumed, was involved in the feeding act. However, it was found that feeding did not take place in the absence

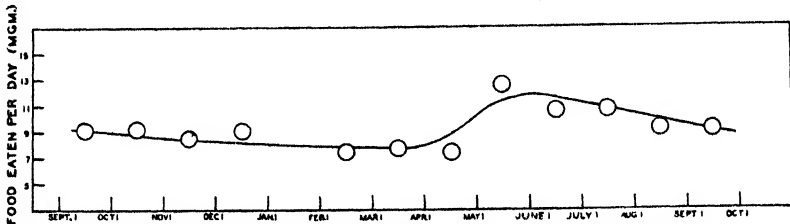


FIGURE 2. Seasonal changes in daily feeding rate by pairs of *Rodolia cardinalis*

of the proper external stimulus. At the laboratory the beetles could not be induced to feed on any insect other than *Icerya purchasi*, no matter how great the degree of starvation. It appears, therefore, that in these experiments the feeding rate was a resultant of the rate of utilization of food consumed, and that both internal and external stimuli entered into the process. The effect of temperature upon feeding, then, was an indirect one, operating through the response of other activities to thermal change. Investigations not yet reported show temperature to have an important influence on the rate of oviposition. The effect of this factor on rate of movement has been demonstrated for other arthropods.^{6 7}

After temperature, season of the year most affects the rate of feeding, as is shown in Figure 2. The rate changed but little from September to April, but it then increased rapidly to a maximum early in June, declining as the summer progressed. Though the season of maximum feeding was also a season of high temperature, the temperature effect has been eliminated statistically. This was also a period of maximum light intensity.

⁴ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., p. 159. Edinburgh and London. 1928.

⁵ CANNON, W. B., and WASHBURN, A. L. AN EXPLANATION OF HUNGER. Amer. Jour. Physiol. 29: 441-454, illus. 1912.

⁶ CROZIER, W. J. ON THE CRITICAL THERMAL INCREMENT FOR THE LOCOMOTION OF A DIPLOPOD. Jour. Gen. Physiol. 7: 123-136, illus. 1924.

⁷ ——— and STIER, T. B. TEMPERATURE CHARACTERISTIC FOR LOCOMOTOR ACTIVITY IN TENT CATERPILLARS. Jour. Gen. Physiol. 7: 123-136, illus.

In analyzing for the effect of age, the life span of each beetle was divided into 10 equal parts, in order to avoid absolute units such as days. The results for the pairs are shown in Figure 3. There was a sharp rise during the first three-tenths, after which the rate fell off slowly with increasing age.

The life span was found to have no significant effect, but the relations between temperature, life span, and food consumption were such that the total quantity of scales eaten per beetle was not a constant under different temperatures. When the total quantity of food was plotted against the mean temperature, the quantity of food eaten was found to be much less at the lower temperatures, although the values are too irregular to permit an exact expression of the relationship.

In estimating the effect of brood on the second residual the means for each brood fell irregularly, showing no significant change in the rate over 11 successive generations reared in the laboratory.

FOUR-HOUR RECORDS

In the second series of experiments the observations were made at shorter intervals. The beetles were caged as before and records

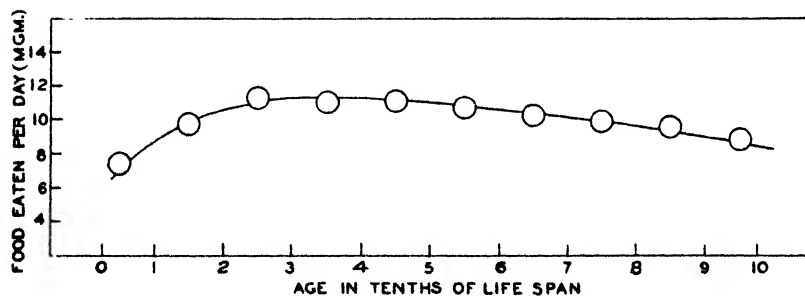


FIGURE 3 — Effect of age on daily feeding rate of pairs of *Rodolia cardinalis*

were taken every four hours. While the daily records were made on individuals bred continuously from the same stock, the 4-hour records were made on two different stocks. The first experiments in this series were made September 20 to 27, 1926, on the line used for daily records. In all, 13 pairs of beetles were used, giving 532 individual values. Beetles for the second set of experiments were taken from an isolated infestation on one bush in the field. Observations were made from March 26 to April 5, 1927, the number of pairs ranging from 9 (first day) to 21, a total of 916 individual records being obtained. Because of the short time covered by these experiments, the only factors considered were temperature and time of day. It was found that the statistical constants for the two sets of tests were quite different, the coefficient for the net regression of food on temperature being 0.171 for the data of 1926 and 0.302 for the data of 1927. Methods of multiple curvilinear correlation were used, and the values for the tests of 1926 were multiplied by the factor 1.32 and added to the data for 1927 to give the curves shown in Figures 4 and 5. The shape of the temperature curve is similar to that for the daily records.

The periodic nature of the feeding is shown in Figure 5. To test the effect of light, experiments were also made under constant light and temperature (25 ± 0.05). The values are shown in Table 1. The

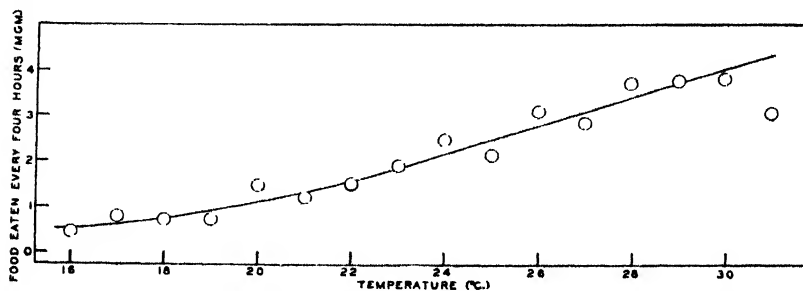


FIGURE 4.—Rate of feeding of *Rodolia cardinalis* as a function of temperature, based on observations of pairs of beetles at 4-hour intervals

probable errors of the means range from 6 to 12 per cent, but the values at 10 a. m. and 2 p. m. may be significantly lower than the rest. In any case it is seen that the curve of Figure 5 was materially altered when light was held constant. The simplest explanation of such an

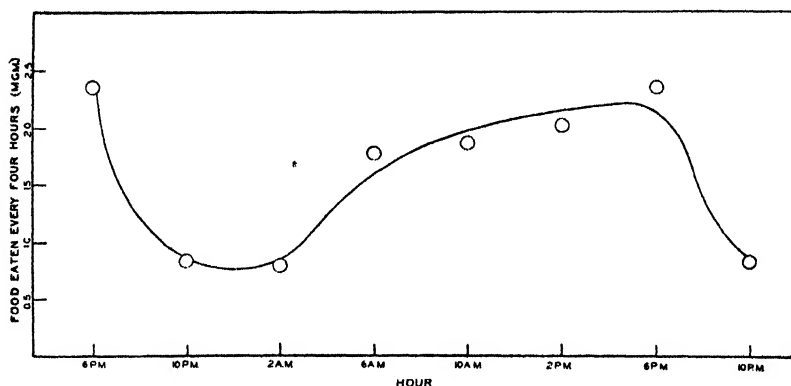


FIGURE 5.—Diurnal fluctuation in the feeding rate of *Rodolia cardinalis*, independent of temperature, based on observations of pairs of beetles at 4-hour intervals

effect would be that in the absence of light the motor activities of the insect were suppressed, resulting in contact with fewer of the external stimuli necessary for feeding. In a qualitative way such an effect on movement has been noted.

TABLE 1.—Diurnal fluctuation in feeding rate of *Rodolia cardinalis* under constant light and temperature

Hour	Average quantity of food eaten
	Mgm.
2 a. m.	1.87±0.12
6 a. m.	1.49±.12
10 a. m.88±.10
2 p. m.93±.11
6 p. m.	1.72±.11
10 p. m.	1.63±.12

An important point in these records is the difference in values of the regression coefficients. Similar differences were found also in the oviposition and developmental rates of the two stocks. It is evident that mathematical constants describing the rates of biological activities for a species are of doubtful validity. Rather do they represent an average of the particular individuals studied, and they may prove to be true of another population only if that population is composed of the same proportion of the same kinds of individuals. Further investigations are being conducted on this point.

SUMMARY

The daily feeding rate of *Rodolia cardinalis* (Muls.) was found to be a function of temperature, age, seasonal change, and sex. Both external and internal stimuli were involved, the quantity of food consumed varying with the rate of egg production.

The effect of temperature was an indirect one, operating through the response to thermal change of activities which condition feeding.

A pronounced seasonal change was observed, the beetles feeding at an increased rate from May through August, as compared with the rate from September to April.

The effect of age was such that the quantity of food consumed is lowest during the first tenth of the life span, rises to a maximum during the third, then shows a slight decrease.

No systematic change was noted in 11 successive broods reared in the laboratory, but a marked difference was found in a stock taken from the field.

Observations made every four hours showed the periodic nature of the feeding process. These diurnal fluctuations were modified under constant light.

VITAMIN G IN CERTAIN MEATS AND MEAT BY-PRODUCTS¹

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INTRODUCTION

The term "vitamin G" is now generally used in the United States to denote the heat-stable, water-soluble vitamin which is necessary for growth in rats and presumably for growth in other animals. In Great Britain the term "vitamin B₂" is used to designate the same vitamin. There is a disposition on the part of some writers to use either one or the other of these terms to denote the antipellagra vitamin also (P-P factor of Goldberger) as though the growth-promoting and antipellagra factors are identical. While in general the two vitamins seem to be similarly distributed, yet their identity has by no means been established, and it seems best at this time not to confuse the two factors by giving them the same name. For this reason the term "vitamin G" is restricted in this paper to the growth-promoting factor.

Since a preliminary experiment by Goldberger and associates² indicated that lean, fresh beef was a fairly good source of growth-promoting vitamin G, it seemed desirable to ascertain the relative values of a number of kinds of meats and meat by-products as sources of this vitamin. The following products have been tested in this laboratory: Beef, pork, lamb, beef liver, pork liver, beef kidney, and beef spleen.

PREVIOUS INVESTIGATIONS

Goldberger and associates³ report the results of an experiment with four rats which indicate that lean beef is a fairly good source of growth-promoting vitamin G. Twenty per cent of dried lean beef as the source of this vitamin in the diet induced moderate growth.

Aykroyd and Roscoe⁴ studied the distribution of vitamin B₂ (vitamin G) in a number of food products, including beef and beef liver, by means of feeding experiments with young rats. Their report indicates that of 17 products tested beef liver was the richest in this vitamin, while beef ranked fourth.

Hoagland and Snider⁵ found that commercial beef extract was a good source of vitamin G. One part of moisture-free beef extract contained approximately as much of this vitamin as three parts of air-dry beef.

¹ Received for publication Mar. 27, 1930; issued August, 1930.

² GOLDBERGER, J. WHEELER, G. A., LILLIE, R. D., and ROGERS, L. N. A FURTHER STUDY OF BUTTER, FRESH BEEF, AND YEAST AS PELLAGRA PREVENTIVES, WITH CONSIDERATION OF THE RELATION OF FACTOR P-P OF PELLAGRA (AND BLACK TONGUE OF DOGS) TO VITAMIN B. Pub. Health Rpts. [U. S.] 41: 297-318, 1926.

³ GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. N. Op. cit.

⁴ AYKROYD, W. R., and ROSCOE, M. R. THE DISTRIBUTION OF VITAMIN B₂ IN CERTAIN FOODS. Biochem. Jour. 23: 483-497, 1929.

⁵ HOAGLAND, R., and SNIDER, G. G. BEEF EXTRACT AS A SOURCE OF VITAMIN G. Jour. Agr. Research 40: 977-990, illus. 1930.

EXPERIMENTAL PROCEDURE

The procedure followed in these experiments was essentially the same, with a few exceptions as that used by the writers in a study of vitamin G in beef extract.⁶ The rats were first fed a basal ration containing corn extract as a source of vitamin B (antineuritic), and lacking only vitamin G, until growth ceased. The rats were then fed this basal ration plus meat or meat by-product as a source of vitamin G. This procedure assured that the rats had exhausted their reserve store of vitamin G, and that the basal ration was practically free from this vitamin before they were fed the test ration. The basal ration was made up as follows:

Constituent	Parts by weight
Casein (N×6.25).....	20
Ash mixture.....	4
Cod-liver oil.....	2
Hydrogenated cottonseed oil.....	8
Corn extract.....	10
Starch to make.....	100

The constituents of the basal ration were prepared in the manner previously described.⁶ When the basal ration was supplemented with meat or meat by-product as the source of vitamin G, the proportion of cottonseed oil was adjusted so that the ration still contained 10 per cent fat. The proportion of casein was also changed so that the test ration contained 20 per cent protein, except when meat protein amounted to 15 per cent or more, when no casein was added. Fifteen per cent of meat protein is ample for optimum growth in rats.

PREPARATION OF MEATS AND MEAT BY-PRODUCTS

The meats and meat by-products were trimmed as free from fat and connective tissue as practicable, ground, and dried at approximately 60° C. in an oven with forced draft. The dried products were ground and those containing much fat were thoroughly extracted with ether. All products were analyzed for nitrogen, and fat was determined in those which had not been extracted with ether. Each product was incorporated with the basal ration before being fed to rats. The percentage of meat or meat by-product in a ration is expressed in terms of fat-free air-dry material.

Three lots of beef (round steak), one lot each of pork tenderloin, smoked ham, lamb (shoulder), beef liver, pork liver, beef kidney, and beef spleen were tested for vitamin G.

RESULTS OF EXPERIMENTS

VITAMIN G IN BEEF, PORK, AND LAMB

The results of the experiments with these products are presented in Figures 1 to 6, inclusive, and in Table 1. As regards Figures 1 to 6, inclusive, the growth curves are largely self-explanatory. Attention is called to the fact that the rats made but little or no growth when fed the basal ration alone (broken lines), but when a sufficient quantity of vitamin G was added to the ration in the form of dried meat, there was a quick response in growth.

⁶ HOAGLAND, R., and SNIDER, G. G. Op. cit.

The experiments with beef (figs. 1, 2, and 3) indicate that 25 per cent of each lot of beef furnished sufficient vitamin G for excellent growth in rats. Fifteen per cent of one lot of beef (fig. 2) induced nearly maximum growth. On the whole it appears that 25 per cent of dried beef, possibly somewhat less, supplied ample vitamin G for rapid growth in rats.

Table 1 shows that both the male and female rats which were fed the largest proportion of each lot of beef made excellent gains in weight. With one exception, the male rats receiving 25 per cent of dried beef made an average daily gain of 3.37 gm. or more during a period of 60 days, the maximum daily gain being 3.85 gm. The average daily gains made by the female rats which were fed the same proportion of beef ranged from 1.84 to 2.77 gm.

Pork tenderloin and smoked ham seemed to be fully as good sources of vitamin G (figs. 4 and 5) as the samples of beef previously reported. Fifteen per cent of dried pork tenderloin and 20 per cent of dried smoked ham, respectively, induced excellent growth in rats. Apparently the tenderloin contained somewhat more vitamin G than the smoked ham.

Table 1 indicates that the rats receiving the larger proportion of tenderloin and ham made excellent growth. The gains made by the male rats ranged from 3.28 to 4.12 gm., and those by the female rats from 1.85 to 2.37 gm.

TABLE 1.—Record of growth and feed consumption by rats fed beef, pork, and lamb as sources of vitamin G

Source of vitamin G in ration	Rat No	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total animal tissue consumed	Average daily intake of animal tissue
			Days	Grams	Grams	Grams	Grams	Grams
15 per cent beef, No. 1764 *	1009-A	Male	56	84	1.50	425	63.75	1.14
	1009-C	do	56	23	.41	244	36.60	.65
	1009-E	do	60	117	1.95	546	81.90	1.36
	1009-D	Female	56	47	.84	280	42.00	.75
	1009-F	do	56	54	.96	382	57.30	1.02
	1010-A	Male	60	166	2.77	524	104.80	1.75
20 per cent beef, No. 1764	1010-C	do	60	129	2.15	426	85.20	1.42
	1010-E	do	60	93	1.55	416	83.20	1.39
	1010-B	Female	60	128	2.13	529	105.80	1.76
	1010-D	do	60	93	1.55	444	88.80	1.48
	1010-F	do	60	108	1.80	471	94.20	1.57
	1018-A	Male	60	120	2.00	513	128.25	2.14
25 per cent beef, No. 1764	1018-C	do	60	224	3.73	688	172.00	2.87
	1018-E	do	60	231	3.85	747	186.75	3.11
	1018-B	Female	60	136	2.27	532	133.00	2.22
	1018-D	do	60	134	2.23	492	123.00	2.05
	1018-F	do	63	116	1.84	559	139.75	2.22
	1031-A	Male	59	206	3.49	717	107.55	1.82
15 per cent beef, No. 1795	1031-C	do	59	96	1.63	514	77.10	1.31
	1031-E	do	59	181	3.07	598	89.70	1.52
	1031-B	Female	62	106	1.71	549	82.35	1.33
	1031-D	do	59	122	2.07	568	89.70	1.52
	1031-F	do	59	128	2.17	593	88.95	1.51
	1032-A	Male	61	227	3.72	776	194.00	3.18
25 per cent beef, No. 1795	1032-C	do	62	228	3.68	806	201.50	3.25
	1032-E	do	61	216	3.54	771	192.75	3.16
	1032-B	Female	61	119	1.95	623	155.75	2.55
	1032-D	do	62	120	1.94	540	135.00	2.18
	1032-F	do	61	158	2.59	663	165.75	2.72
	1045-A	Male	59	99	1.68	436	62.61	1.06
14.36 per cent beef, No. 1824	1045-B	do	59	158	2.68	572	82.14	1.39
	1045-C	do	60	146	2.43	593	85.15	1.42
	1045-D	do	60	112	1.87	493	69.36	1.16
	1045-E	do	60	112	1.87	500	71.60	1.20
	1045-F	Female	60	135	2.25	571	82.00	1.37

* Same numbers refer to different lots of meat used in the experiment.

TABLE 1.—Record of growth and feed consumption by rats fed beef, pork, and lamb as sources of vitamin G—Continued

Source of vitamin G in ration	Rat No	Sex	Duration of test, Days	Total gain in weight, Grams	Average daily gain in weight, Grams	Total feed consumed, Grams	Total animal tissue consumed, Grams	Average daily intake of animal tissue, Grams
19 23 per cent beef, No. 1824	1046-A	Male	59	154	2.61	577	110.96	1.88
	1046-D	do.	60	148	2.47	474	91.15	1.52
	1046-E	do.	60	142	2.37	532	102.30	1.71
	1046-B	Female	59	144	2.44	551	105.96	1.80
	1046-F	do.	60	67	1.12	308	59.23	.99
25 per cent beef, No. 1824	1055-A	Male	49	165	3.37	422	105.50	2.15
	1055-C	do.	45	166	3.69	477	119.25	2.65
	1055-E	do.	49	171	3.49	491	122.80	2.51
	1055-B	Female	39	108	2.77	372	93.00	2.38
	1055-D	do.	41	102	2.49	354	88.50	2.16
	1055-F	do.	35	97	2.77	335	83.80	2.39
10 per cent pork tenderloin, No. 1768.	1008-A	Male	60	149	2.48	579	57.9	.96
	1008-C	do.	60	170	2.83	592	59.2	.99
	1008-B	Female	60	92	1.53	439	43.9	.73
	1008-D	do.	60	54	.90	361	36.1	.60
	1008-E	do.	60	110	1.83	473	47.3	.79
	1008-F	do.	60	96	1.60	476	47.6	.79
15 per cent pork tenderloin, No. 1768.	1012-A	Male	60	205	3.42	701	105.15	1.75
	1012-B	do.	60	201	3.35	665	99.75	1.66
	1012-F	do.	57	187	3.28	615	92.25	1.62
	1012-C	Female	60	134	2.23	563	84.45	1.41
	1012-D	do.	60	123	2.05	544	81.60	1.36
	1012-E	do.	60	137	2.28	525	78.75	1.31
10 per cent smoked ham, No. 1773.	1017-A	Male	60	152	2.53	408	40.80	.68
	1017-B	do.	60	106	1.77	399	39.90	.66
	1017-C	do.	60	61	1.02	317	31.70	.53
	1017-D	do.	60	130	2.17	433	43.30	.72
	1017-F	do.	61	114	1.87	455	45.50	.74
	1017-E	Female	60	115	1.92	497	49.70	.83
15 per cent smoked ham, No. 1773.	1019-A	Male	60	138	2.30	500	75.00	1.25
	1019-C	do.	60	165	2.75	595	89.25	1.49
	1019-B	Female	60	125	2.08	529	79.35	1.32
	1019-D	do.	60	110	1.83	506	75.90	1.26
	1019-F	do.	63	118	1.87	612	91.80	1.46
20 per cent smoked ham, No. 1773.	1030-A	Male	60	209	3.48	776	155.20	2.59
	1030-B	do.	60	247	4.12	803	160.60	2.68
	1030-E	do.	60	205	3.42	726	145.20	2.42
	1030-C	Female	60	142	2.37	616	123.20	2.05
	1030-D	do.	60	135	2.25	594	118.80	1.98
	1030-F	do.	60	111	1.85	572	114.40	1.91
15 per cent lamb	1089-A	Male	60	189	3.15	700	105.00	1.75
	1089-C	do.	60	166	2.77	635	95.25	1.59
	1089-B	Female	60	120	2.00	591	88.65	1.48
	1089-D	do.	60	101	1.68	512	76.80	1.28
	1089-E	do.	60	122	2.03	638	95.70	1.60
	1089-F	do.	60	144	2.40	667	100.05	1.67
25 per cent lamb	1090-A	Male	60	285	4.75	888	222.00	3.70
	1090-C	do.	60	235	3.92	754	188.50	3.14
	1090-B	Female	60	143	2.38	585	146.25	2.44
	1090-D	do.	60	131	2.18	604	151.00	2.52
	1090-E	do.	60	111	1.85	581	145.25	2.42
	1090-F	do.	60	123	2.05	584	146.00	2.43

Lamb (fig. 6) seemed to contain fully as much vitamin G as the samples of beef and pork previously examined. Fifteen per cent of dried lamb induced nearly as good growth in rats as 25 per cent of the same product.

From Table 1 the average daily gains made by two male rats fed 15 per cent lamb were 2.77 and 3.15 gm., while two male rats fed 25 per cent gained 3.92 and 4.75 gm. The female rats receiving 15 per cent lamb gained from 1.68 to 2.40 gm., and those fed 25 per cent gained from 1.85 to 2.38 gm. daily.

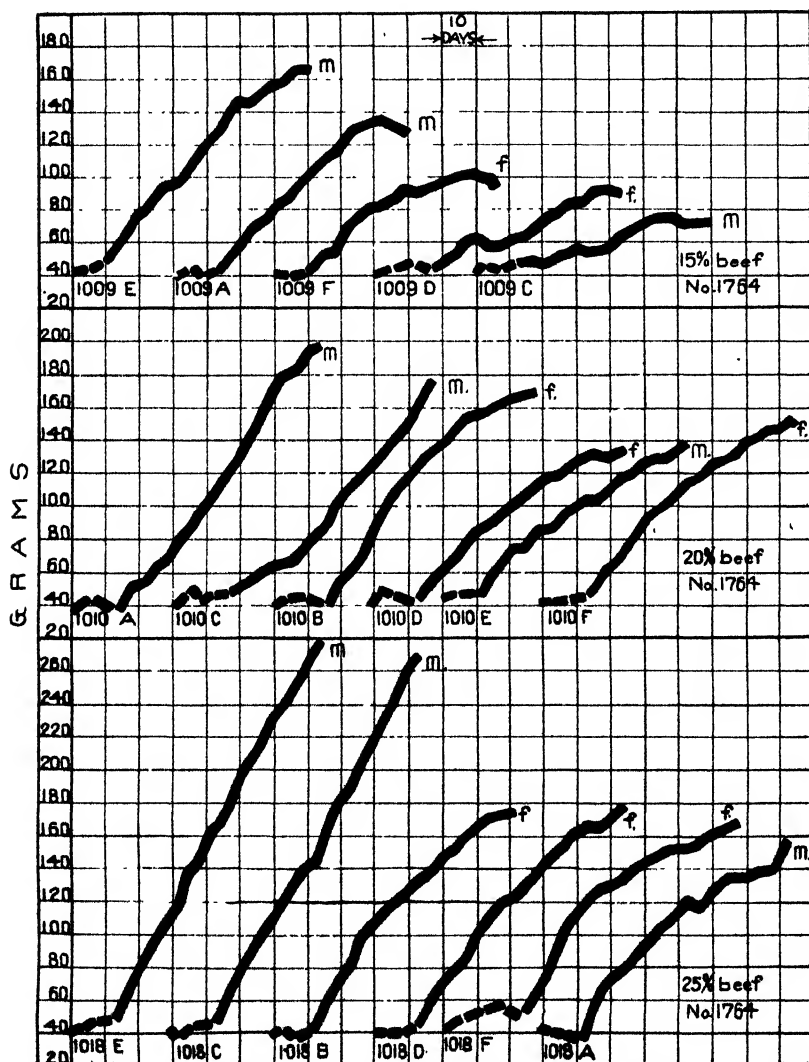


FIGURE 1.—Growth of rats fed rations containing different percentages of the same lot of fat-free dried beef as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after beef had been added to the basal ration as a source of vitamin G.

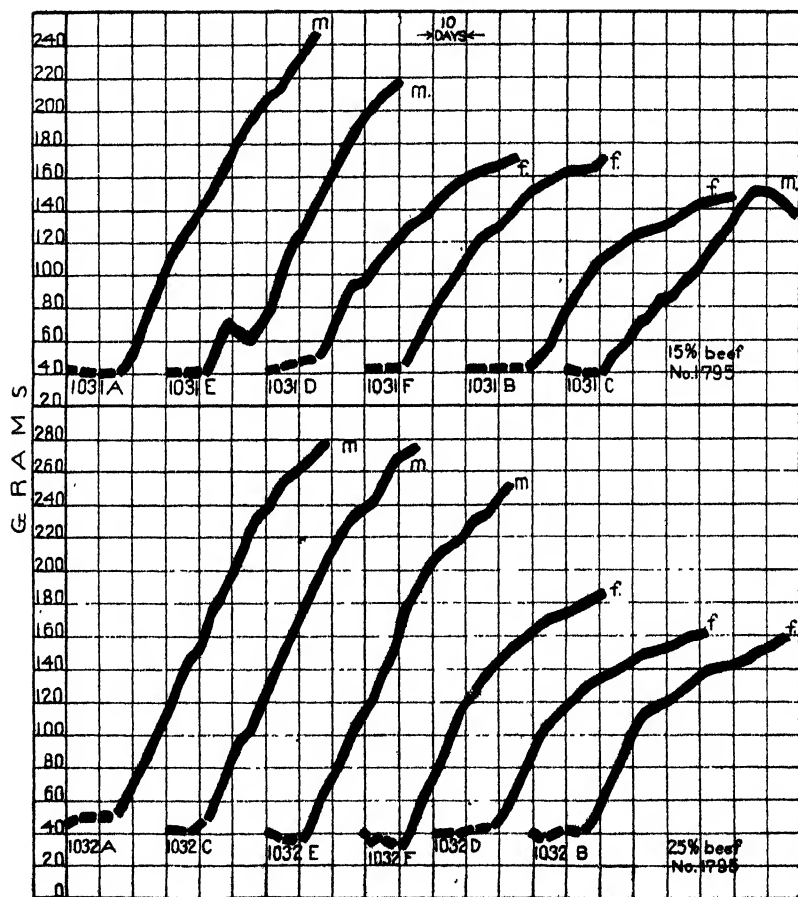


FIGURE 2.—Growth of rats fed rations containing different percentages of the same lot of fat-free dried beef as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after beef had been added to the basal ration as a source of vitamin G

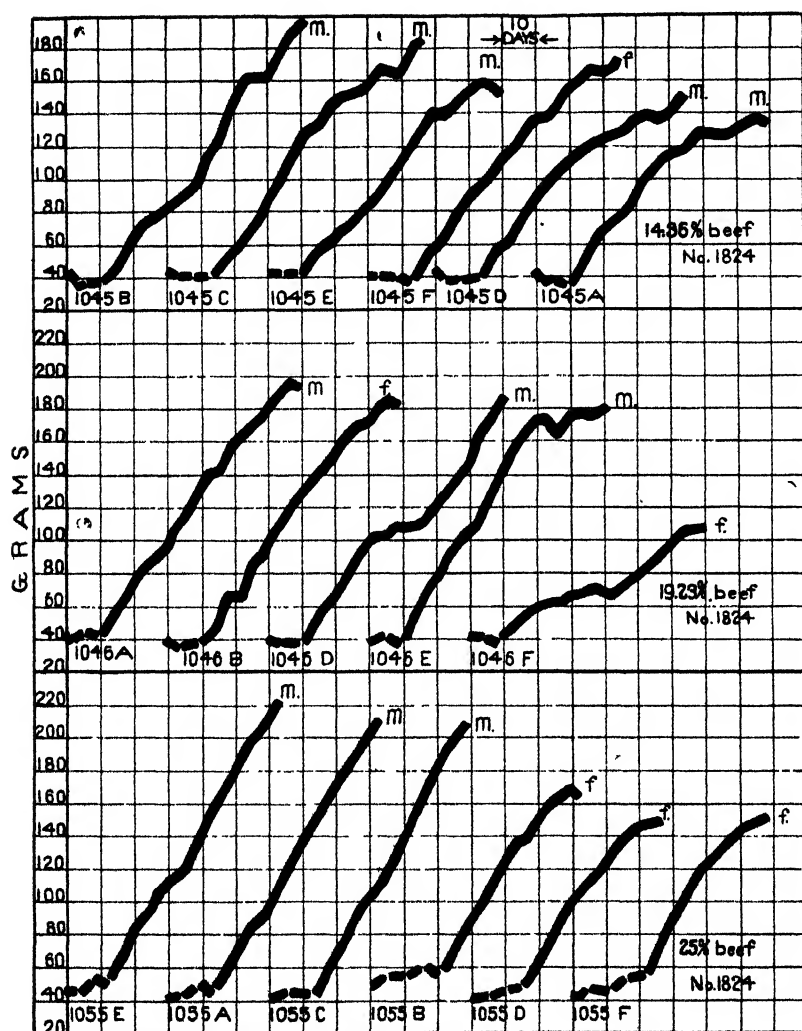


FIGURE 3.—Growth of rats fed different percentages of fat-free dried beef as a source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines indicate growth after beef has been added to the basal diet as a source of vitamin G

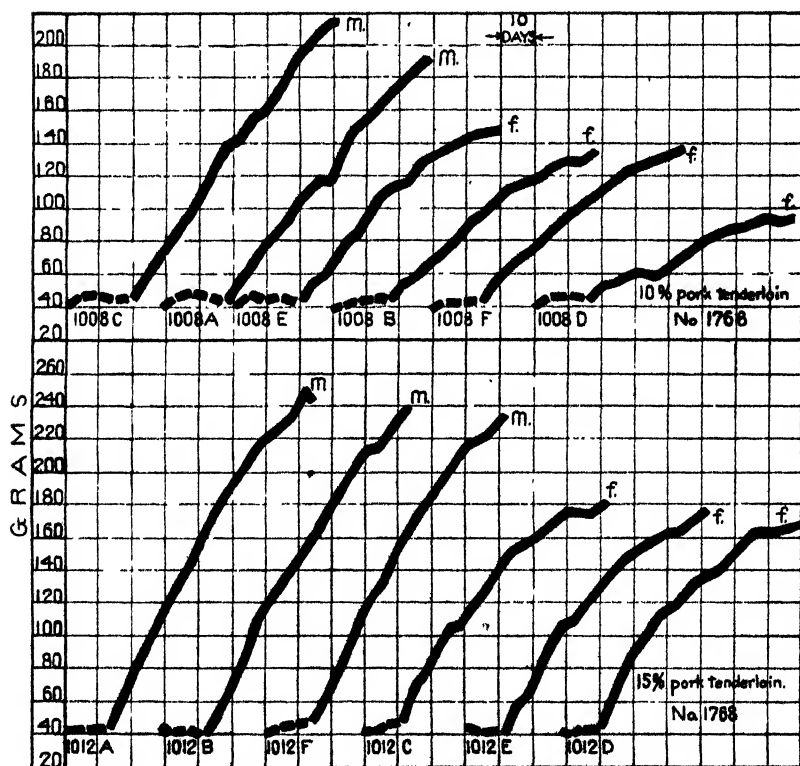


FIGURE 4.—Growth of rats fed different percentages of fat-free dried pork tenderloin as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried pork had been added to the basal ration as a source of vitamin G.

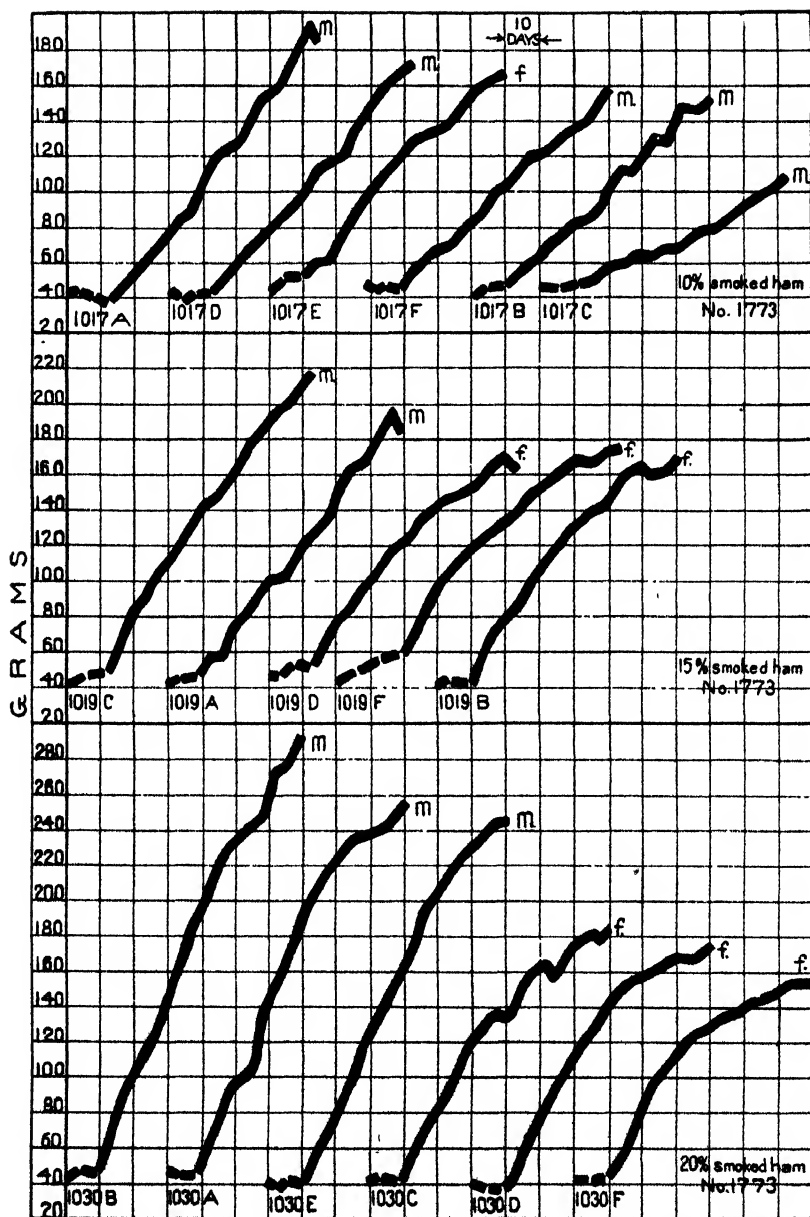


FIGURE 5.—Growth of rats fed different percentages of fat-free dried smoked ham as the source of vitamin G. The broken lines indicate growth upon the basal ration alone, the solid lines denote growth after dried pork had been added to the basal ration as a source of vitamin G

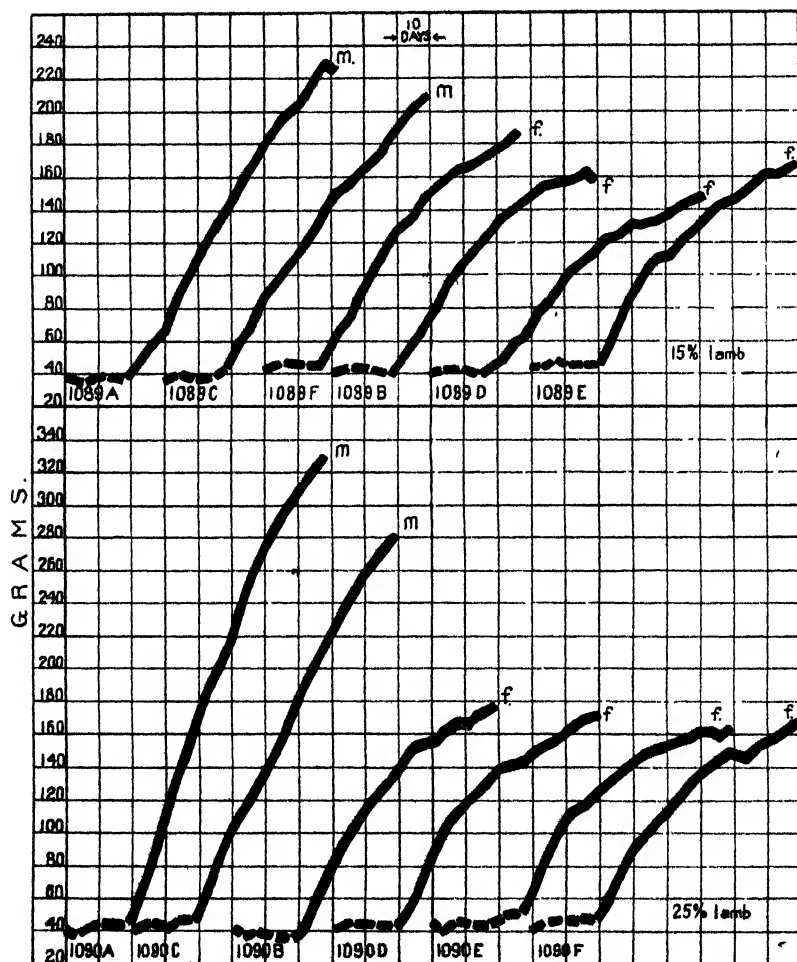


FIGURE 6.—Growth of rats fed different percentages of fat-free dried lamb as the source of vitamin G. The broken lines indicate growth upon the basal ration alone which was practically free from vitamin G; the solid lines denote growth after dried lamb had been added to the basal diet as a source of vitamin G.

VITAMIN G IN BEEF SPLEEN, BEEF LIVER, PORK LIVER, AND BEEF KIDNEY

One lot of each of the above-mentioned products was tested for vitamin G by feeding experiments with rats. In Figure 7 are shown the results obtained when 10 and 15 per cent, respectively, of dried beef spleen furnished vitamin G in the diet. Apparently 15 per cent of dried spleen supplied sufficient vitamin G for excellent growth in rats. Table 2 shows that the average daily gains made by the male rats fed 15 per cent spleen ranged from 2.98 to 3.45 gm., and those for the female rats ranged from 1.77 to 2.03 gm.

TABLE 2.—Record of growth and feed consumption by rats fed beef spleen, beef liver, pork liver, and beef kidney as sources of vitamin G

Source of vitamin G in ration	Rat No.	Sex	Dura- tion of test	Total gain in weight	Average daily gain in weight	Total feed con- sumed	Total animal tissue consumed	Average daily in- take of animal tissue
			Days	Grams	Grams	Grams	Grams	Grams
10 per cent beef spleen, No. 1805	1035-A	Male.....	60	148	2.47	574	57.40	0.90
	1035-Bdo.....	60	82	1.37	323	32.30	.54
	1035-Cdo.....	60	130	2.17	470	47.00	.78
	1035-D	Female.....	60	117	1.95	515	51.5	.86
	1035-Edo.....	60	119	1.98	490	49.0	.82
	1035-Fdo.....	60	97	1.62	507	50.7	.84
15 per cent beef spleen, No. 1805	1037-A	Male.....	60	179	2.98	625	94.20	1.57
	1037-Bdo.....	60	201	3.35	672	100.80	1.68
	1037-Cdo.....	60	207	3.45	681	102.15	1.70
	1037-D	Female.....	60	108	1.80	496	74.40	1.24
	1037-Edo.....	60	106	1.77	515	77.55	1.29
	1037-Fdo.....	60	122	2.03	464	69.60	1.16
1.36 per cent beef liver, No. 1800	1056-A	Male.....	60	159	2.65	539	7.33	.12
	1056-Cdo.....	60	204	3.40	649	8.83	.15
	1056-Edo.....	60	174	2.90	581	7.90	.13
	1056-B	Female.....	60	117	1.95	409	5.56	.09
	1056-Ddo.....	60	113	1.88	465	6.32	.10
	1056-Fdo.....	60	117	1.95	479	6.51	.11
3 per cent beef liver, No. 1800	1043-A	Male.....	60	200	3.33	662	19.86	.33
	1043-Bdo.....	60	254	4.23	764	22.92	.38
	1043-Cdo.....	60	225	3.75	687	20.61	.34
	1043-Edo.....	60	203	3.38	665	19.95	.33
	1043-D	Female.....	60	138	2.30	523	15.69	.26
	1043-Fdo.....	60	141	2.35	592	17.76	.30
1.38 per cent pork liver, No. 1806	1057-A	Male.....	60	154	2.57	542	7.48	.12
	1057-Cdo.....	60	120	2.00	486	6.71	.11
	1057-Edo.....	60	160	2.67	529	7.30	.12
	1057-B	Female.....	60	119	1.98	482	6.65	.11
	1057-Ddo.....	60	134	2.23	550	7.59	.13
	1057-Fdo.....	60	133	2.22	526	7.26	.12
3.05 per cent pork liver, No. 1806	1042-A	Male.....	60	262	4.37	753	22.97	.38
	1042-Bdo.....	60	192	3.20	624	19.03	.32
	1042-Cdo.....	60	218	3.63	687	20.95	.35
	1042-Edo.....	60	206	3.43	661	20.16	.34
	1042-D	Female.....	60	130	2.17	502	17.14	.28
	1042-Fdo.....	60	149	2.48	555	16.93	.28
1.34 per cent beef kidney...	1074-A	Male.....	60	113	1.88	448	6.00	.10
	1074-Bdo.....	60	139	2.32	462	6.19	.10
	1074-Cdo.....	60	119	1.96	437	5.86	.10
	1074-D	Female.....	60	81	1.35	304	4.88	.08
	1074-Edo.....	60	92	1.53	428	5.74	.10
	1074-Fdo.....	60	98	1.63	380	5.09	.08
2.77 per cent beef kidney...	1069-A	Male.....	60	197	3.28	583	16.15	.27
	1069-Cdo.....	60	233	3.88	654	18.12	.30
	1069-Edo.....	60	223	3.72	616	17.06	.28
	1069-B	Female.....	63	134	2.13	558	15.46	.24
	1069-Ddo.....	63	113	1.85	420	11.77	.20
	1069-Fdo.....	63	135	2.14	523	14.49	.23

The experiments with beef liver, pork liver, and beef kidney as sources of vitamin G in the diets of rats are shown in Figures 8, 9, and 10, and in Table 2. An examination of these figures indicates that

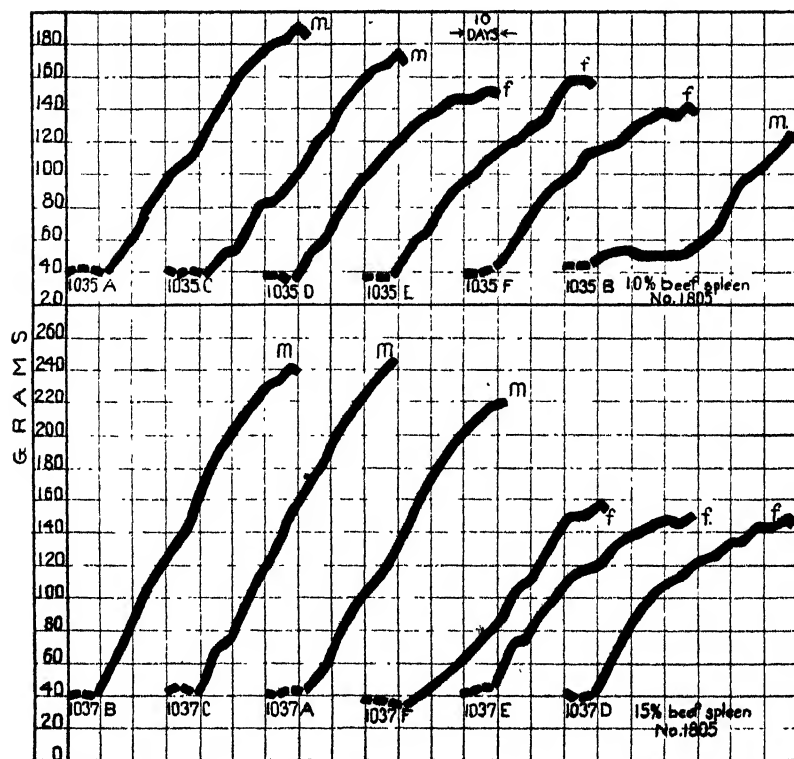


FIGURE 7.—Growth of rats fed different percentages of dried beef spleen as the source of vitamin G. The percentage is expressed in terms of fat-free dried beef spleen. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried beef spleen had been added to the basal diet as a source of vitamin G.

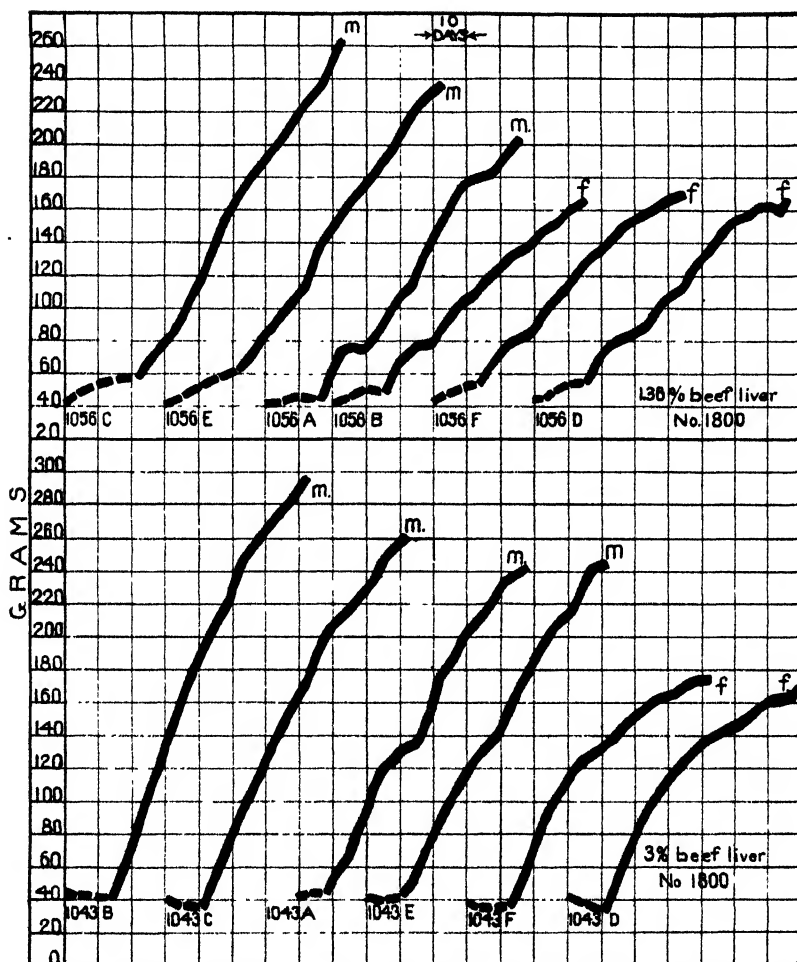


FIGURE 8.—Growth of rats fed different percentages of dried beef liver as the source of vitamin G. The percentage is expressed in terms of fat-free dried liver. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried liver had been added to the basal diet as a source of vitamin G

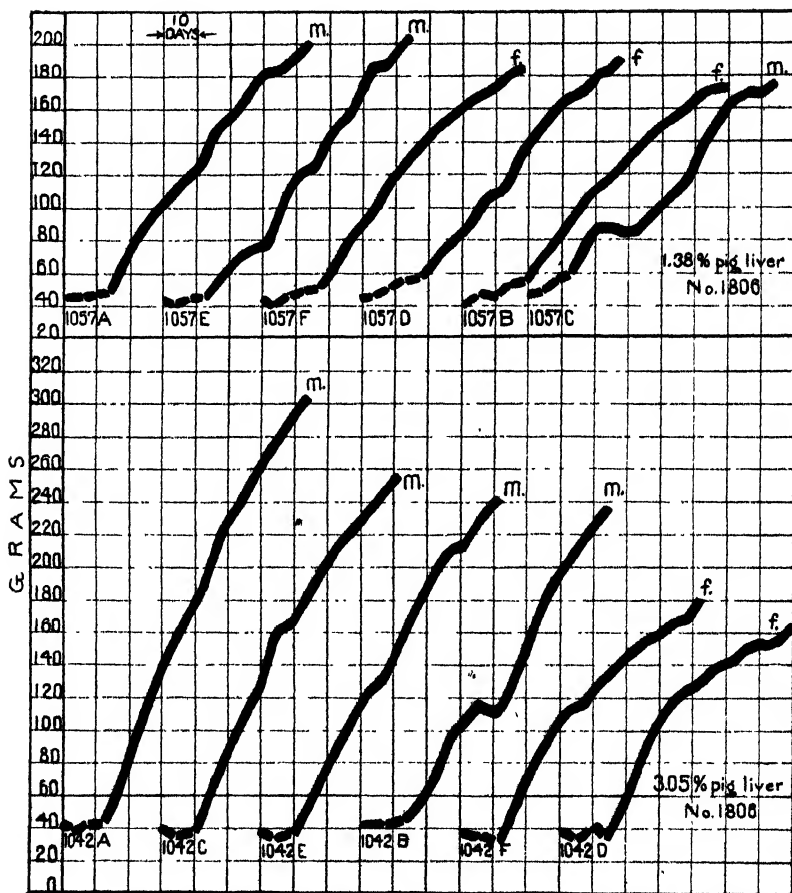


FIGURE 9.—Growth of rats fed different percentages of dried pig liver as the source of vitamin G. The percentage is expressed in terms of fat-free dried liver. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried liver had been added to the basal diet as a source of vitamin G.

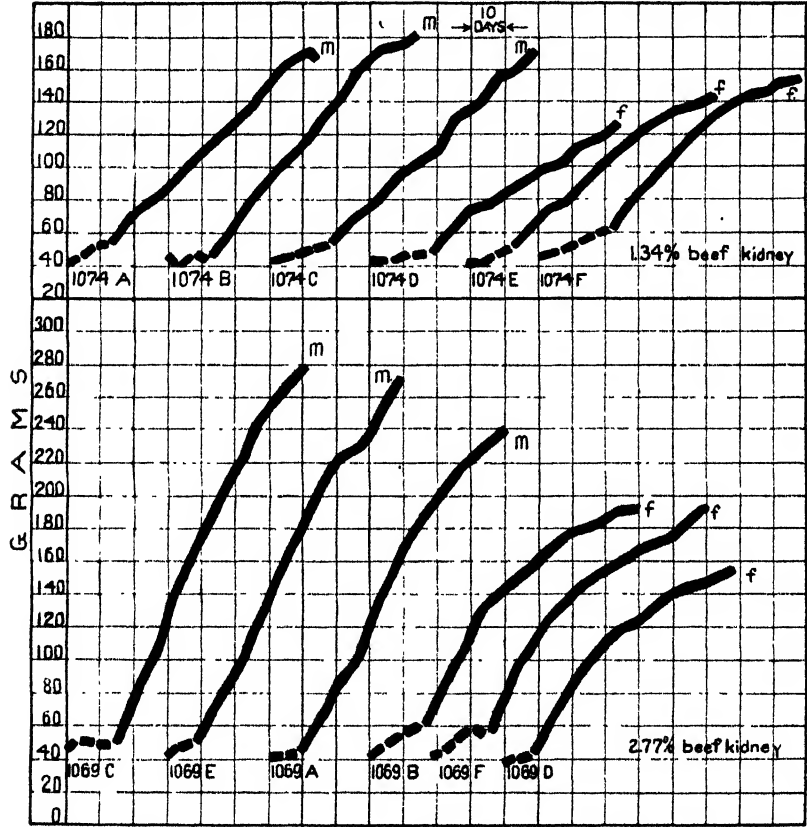


FIGURE 10.—Growth of rats fed different percentages of dried beef kidney as the source of vitamin G. The percentage is expressed in terms of fat-free dried beef kidney. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried beef kidney had been added to the basal diet as a source of vitamin G

liver and kidney were very much richer in vitamin G than lean meat. Thus, as little as 1.38 per cent of dried beef liver or dried pork liver induced very good growth in rats, and 3 to 3.05 per cent of the same products promoted excellent growth. Beef kidney when it constituted 1.34 per cent of the diet, did not seem to be quite so good a source of vitamin G as liver, yet 2.77 per cent of the dried kidney induced rapid growth.

Table 2 shows that male rats which were fed approximately 3 per cent of dried beef or dried pork liver as a source of vitamin G made average daily gains ranging from 3.20 to 4.37 gm., and that female rats fed the same rations made gains ranging from 2.17 to 2.48 gm. Average daily gains made by male rats fed 2.77 per cent dried beef kidney ranged from 3.28 to 3.88 gm., and the gains made by the female rats fed the same rations ranged from 1.88 to 2.14 gm.

SUMMARY AND CONCLUSIONS

In this paper are reported the results of feeding experiments with young albino rats to determine the relative quantities of growth-promoting vitamin G in beef, pork, lamb, beef spleen, beef liver, pork liver, and beef kidney. Comparisons are made on the basis of air-dry, fat-free material.

Beef, pork, and lamb appeared to contain approximately the same quantities of vitamin G, allowance being made for some variation in the vitamin content of different lots of the same kind of meat. From 15 to 25 per cent of dried beef, pork, or lamb furnished sufficient vitamin G for excellent growth in rats.

Beef spleen contained approximately as much vitamin G as beef.

Beef liver, pork liver, and beef kidney were found to be rich sources of vitamin G. Apparently these products contained approximately five to eight times as much of this vitamin as beef, pork, or lamb. Three per cent of beef liver, 3.05 per cent of pork liver, and 2.77 per cent of beef kidney, respectively, furnished an ample supply of vitamin G for rapid growth in rats. The minimum quantity necessary for normal growth is probably considerably less than the proportions indicated.

RELATION BETWEEN THE VIGOR OF THE CORN PLANT AND ITS SUSCEPTIBILITY TO SMUT (*USTILAGO ZEAE*)¹

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INTRODUCTION

A number of investigators have found that the proportion of smutted corn is usually greater on more fertile than on less fertile soil. Some appear to have held the view that the increased smut on fertile soil is due to more favorable conditions favoring spore production. Hitchcock and Norton² state that

smut is usually more abundant when the soil has been recently manured * * *. Since manure forms a favorable breeding ground for smut, fresh manure should not be applied to corn ground, especially in damp soil.

Others who may have in part subscribed to this view associate susceptibility with a tender, succulent, vigorously growing condition of the corn tissues.

Selby and Hickman,³ referring to the smutting of corn, noted that "sod ground or manured land may be expected to show more smut than corn stubble." Arthur and Stuart⁴ have written that

another way in which the plant is made more liable to infection is by increase in the amount of tender growing tissue. This may be brought about by more luxuriant growth, due to greater water or food supply.

Piemeisel⁵ stated that "a moist, rich soil produces a very vigorous succulent plant which apparently is also more susceptible to smut."

After investigating the vegetative vigor of the host plant as a factor influencing susceptibility or resistance to certain rust diseases of the higher plants, Raines⁶ states:

* * * the vegetative vigor of the host and the virulence of the disease may be in direct relation. * * * a more catholic point of view in pathological thought, recognizing that, for longer or shorter phases in the course of a disease, the relation between host and parasite may be highly mutualistic, would be of material value as a working concept in the study of disease and in defining the practical problem of disease prevention and control.

Since yield is one expression of vigor, it is desirable that the corn breeder, seeking increased yield through the selection of resistant strains, should have a clear understanding of the relations between host vigor and susceptibility to the smut of corn. Most corn breeders are rejecting seed of smutted plants on general principles except for unusual reasons. If vigor and susceptibility are directly associated

¹ Received for publication Apr. 25, 1930; issued August, 1930.

² HITCHCOCK, A. S., and NORTON, J. B. S. CORN SMUT. Kans. Agr. Expt. Sta. Bul. 62, p. 169-212, illus. 1906.

³ SELBY, A. D., and HICKMAN, J. F. CORN SMUT. Ohio Agr. Expt. Sta. Bul. 78: 92-96. 1897.

⁴ ARTHUR, J. C., and STUART, W. CORN SMUT. Ind. Agr. Expt. Sta. Ann. Rpt. (1898/99) 12: 84-135, illus. 1900.

⁵ PIEMEISEL, F. J. FACTORS AFFECTING THE PARASITES OF *USTILAGO ZEAE*. Phytopathology 7: [294]-307. 1917.

⁶ RAINES, M. A. VEGETATIVE VIGOR OF THE HOST AS A FACTOR INFLUENCING SUSCEPTIBILITY AND RESISTANCE TO CERTAIN DISEASES OF THE HIGHER PLANTS. II. Amer. Jour. Bot. 9: 215-238, illus. 1922.

it seems that the general tendency of such practice may be to produce less vigorous and possibly less productive corn. The data that follow are offered as a contribution in the solution of this problem.

METHODS AND MATERIAL

The experiments herein reported were conducted on the Arlington Experiment Farm, Rosslyn, Va., in 1925, 1926, and 1927. Corn lines that had been selfed 6 to 11 years and F_1 crosses between such lines were used. Field comparisons were usually made between short single-row plots, repeated in systematic order as often as the quantity of available seed would permit, ranging for the different tests from six to twenty-one times. Exceptions to this general rule are noted in the text.

Natural infection was relied upon to produce the disease. It is believed that there was abundant opportunity for plants to become smutted, as from 60 to 100 per cent of the plants of extremely susceptible strains grown in plots adjacent to the experimental plots were diseased. Only the nodal smut infections are reported here. Nearly all, if not all, of these originated in lateral buds or potential ear shoots. The infection of leaves and other plant parts was of very minor importance in these experiments.

Vigor in these experiments was measured by relative size or by weight of total plant growth produced per average unit of time or by weight of grain produced by plants requiring about the same time to mature. All weights were determined on uniformly air-dry material.

DIFFERENCES IN DEGREE OF VIGOR AND THE RELATIVE NUMBER OF SMUTTED PLANTS

DIFFERENCES BETWEEN F_1 CROSSES AND THEIR SELFED PARENTS

In 1925 two relatively resistant selfed lines and the F_1 cross between them and two relatively susceptible selfed lines and the F_1 cross between them were compared for their reaction to smut. Each line and each cross was grown in 20 distributed 10-plant rows. The percentage of smutted plants in each and the excess percentage of smut in the crossover that in the more susceptible of the two parents are shown in Table 1. The cross of the resistant lines had 1.3 ± 0.80 per cent and the cross of the susceptible lines had 10.7 ± 1.89 per cent more smutted plants than the more susceptible parent. As each cross matured as early as either of its parents and the amount of plant growth was much greater, the vigor as expressed by the average growth rate also was greater. Vigor and the percentage of smutted plants, therefore, tended to be directly associated in both the resistant and the susceptible corn. The difference in the resistant corn, however, was not significant statistically.

DIFFERENCES BETWEEN PLANTS OF THE SAME F_1 CROSSES

In 1926 seed of 21 crosses was planted, each cross being placed in two adjacent rows. The seed was dropped 3 inches apart and the seedlings were thinned to the usual stand five weeks after planting. In thinning, the larger seedlings were left in the first row of each cross and the smaller ones were left in the second row. Field observations showed that these relative differences in plant size tended to be con-

tinuous throughout the season. When the plants were mature there were 241 from the larger seedlings and 239 from the smaller ones. In the first class there were 54 smutted nodes and 33 smutted ears per 1,000 plants. In the second class there were 25 smutted nodes and 17 smutted ears per 1,000 plants. In other words, 116 per cent more nodal smut and 94.1 per cent more ear smut were associated with the larger than with the smaller plants. The corn from the larger seedlings, as a whole, matured in slightly less time. Its vigor, as measured by average growth per day, therefore, was certainly greater than that of the plants from the smaller seedlings.

TABLE 1.—*Relative proportions of smutted plants in selfed lines and in crosses from them as grown at Arlington Experiment Farm, Rosslyn, Va., in 1925*

Selfed lines and F ₁ crosses between them *	Smutted plants	Excess over that of the more susceptible of the two parents
	Per cent	Per cent
Resistant:		
C. I. 207 F-54	2.2±0.478	1.3±0.80
C. I. 207 F-54 × C. I. 218 F-79	3.7±.596	
C. I. 218 F-79	2.4±.537	
Susceptible:		
C. I. 207 F-90	67.8±.854	10.7±1.89
C. I. 207 F-90 × C. I. 218 F-69	91.9±1.257	
C. I. 218 F-69	81.2±1.417	

* Numbers preceded by C. I. are accession numbers of the Office of Cereal Crops and Diseases, and numbers preceded by F are self-fertilized lines of the accession.

DIFFERENCES DUE TO DIFFERENT METHODS OF CULTURE

In 1927 two 6-row plots 132 feet long of furrow-planted corn alternating with similar plots of level-planted corn were grown. The two outside rows of each plot were guards. The four inner rows consisted of two adjacent rows of each of two crosses. Each row was treated as a replication. The furrow-planted corn was planted in furrows that had been opened with a lister and was left without cultivation for the first 73 days after planting. The furrows then were filled by cultivation to the level of the land in the other plots, and subsequent cultivation was the same as that for the others. The level-planted corn was cultivated in the normal way.

The relation between the degree of smut infection and the average growth rate during the 73 days following planting for two F₁ crosses, 37×86 and 49×99, and for both treatments is shown in Table 2. The numbers of smut galls recorded are those on all the plants in each replication, and the plant weights are the average weights of all plant growth above the ground for 10 representative plants from each replication. For cross 37×86 the average height of the furrow-planted corn at this time was 38.4 and of the level-planted corn 46.3 inches. For cross 49×99 the height of the furrow-planted corn was 44.5 and of the level-planted corn 49.5 inches.

Many of the furrow-grown plants were distinctly lighter green than those in the level planting. The plants in the level-planted plots had more smut galls per 1,000 plants than those in the furrow-planted plots in seven of the eight comparisons. The average number of smut galls per 1,000 on the level-planted corn was 2.5 times

that on the furrow-planted corn for cross 37×86 and 1.8 times for cross 49×99. The average plant weight and the average daily gain in weight in the level-planted plots were greater than those in the furrow-planted plots in seven of the eight comparisons. The average daily weight increase of the level-planted corn was 1.7 times that of the furrow-planted corn for cross 37×86 and 1.5 times for cross 49×99.

TABLE 2.—*Relation between degree of smut infection and average growth rate of two F₁ corn crosses during the 73 days following planting under furrow and level culture on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Item	Furrow-planted corn in—					Level-planted corn in—				
	Row 1	Row 2	Row 3	Row 4	All rows	Row 1	Row 2	Row 3	Row 4	All rows
Cross 37×86:										
Total number of plants.....	89	90	87	97	363	70	67	77	71	285
Total number of smut galls.....	9	9	21	28	67	31	38	34	29	132
Number of smut galls per 1,000 plants.....	101	100	241	289	185	443	567	442	408	463
Average plant weight (grams).....	45.7	43.9	59.4	44.1	48.3	80.0	78.9	89.7	86.3	83.7
Average daily weight increase (grams).....	.63	.60	.81	.60	.66	1.10	1.08	1.23	1.18	1.15
Cross 49×99:										
Total number of plants.....	85	95	84	83	347	76	75	68	73	292
Total number of smut galls.....	1	0	0	5	6	3	3	2	1	9
Number of smut galls per 1,000 plants.....	12	0	0	60	17	39	40	29	14	31
Average plant weight (grams).....	35.3	44.7	46.4	66.2	48.2	81.0	65.6	72.7	66.1	71.4
Average daily weight increase (grams).....	.48	.61	.64	.91	.66	1.11	.90	1.00	.91	.98

The relation between the degree of smut infection and the average growth rate of the two F₁ crosses, 37×86 and 49×99, during the period between planting and silking for the two types of planting is shown in Table 3. The numbers of smut galls are the totals for all the plants in each replication, and the plant weights are the average weights of all plant growth aboveground for 10 representative plants from each replication.

TABLE 3.—*Relation between degree of smut infection and average growth rate of two F₁ corn crosses during the period between planting and silking under furrow and level culture on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Data	Furrow-planted corn in—					Level-planted corn in—				
	Row 1	Row 2	Row 3	Row 4	All rows	Row 1	Row 2	Row 3	Row 4	All rows
Cross 37×86:										
Total number of plants.....	76	76	73	81	306	51	48	57	60	216
Total number of smut galls.....	8	5	9	12	34	22	13	24	31	90
Number of smut galls per 1,000 plants.....	105	66	123	148	111	431	271	421	517	417
Average number of days from planting to silking.....	110	110	105	109	108.5	105	106	104	102	104.3
Average plant weight (grams).....	188.5	195.0	257.7	204.9	211.5	310.5	328.1	355.7	385.2	347.4
Average daily weight increase (grams).....	1.71	1.77	2.45	1.88	1.95	2.96	3.19	3.42	3.78	3.33
Cross 49×99:										
Total number of plants.....	74	86	74	73	307	66	64	59	64	253
Total number of smut galls.....	3	4	4	5	16	7	4	11	10	32
Number of smut galls per 1,000 plants.....	41	47	54	68	52	106	63	186	156	126
Average number of days from planting to silking.....	106	104	101	102	103.3	102	101	101	101	101.3
Average plant weight (grams).....	178.9	201.9	266.5	296.7	236.0	324.8	348.0	398.5	345.7	339.3
Average daily weight increase (grams).....	1.69	1.94	2.64	2.91	2.28	3.18	3.45	3.95	3.42	3.35

For cross 37×86 the average height of the furrow-planted corn was 89.8 and of the level-planted corn 104.2 inches. For cross 49×99 the height of the furrow-planted corn was 103.8 and of the level-planted corn 114.8 inches. For the first cross the average number of days from planting to silking required by the furrow-planted corn was 108.5 and by the level-planted corn 104.3. For the second cross the average number of days from planting to silking for the furrow-planted corn was 103.3 days and for the level-planted corn 101.3 days.

The level-planted corn had more smut galls per 1,000 plants than the furrow-planted corn in all eight comparisons. The average number of smut galls per 1,000 plants on level-planted corn was 3.8 times that on the furrow-planted corn for cross 37×86 and 2.4 times for cross 49×99.

The average plant weight and the average daily weight increase on the level-planted plots were greater than those on the furrow-planted plots in all comparisons. The average daily weight increase of the level-planted corn was 1.7 times that of the furrow-planted corn for cross 37×86 and 1.5 times that of the furrow-planted corn for cross 49×99.

The relation between the degree of smut infection and the average growth rate of the F_1 corn cross 37×49 during the entire growth period for the two methods of planting is shown in Table 4. Each datum is the average of those from 10 plants. The 60 plants of furrow-planted corn and the 60 plants of level-planted corn were so selected that a plant started in a furrow stood opposite a plant started in an adjacent row on level land. All plants grew in a normal stand. The average growth period for the furrow planting was 153 days and for the level planting 150 days.

TABLE 4.—*Relation between degree of smut infection and average growth rate of an F_1 corn cross, 37×49, during the entire growth period for furrow and level planting on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Number of smut galls per 1,000 plants—		Average daily weight increase in—			
		Mature culms on plants—		Plant yield of grain from plants—	
Furrow planted	Level planted	Furrow planted	Level planted	Furrow planted	Level planted
		<i>Gram</i>	<i>Gram</i>	<i>Grams</i>	<i>Grams</i>
100	300	0.82	0.82	2.07	2.45
0	300	.57	.82	1.61	2.00
100	300	.70	.90	1.99	2.85
0	100	.53	.81	1.38	2.11
0	200	.52	.71	1.39	1.76
100	600	.63	.78	1.80	1.94
Av. 50	300	.63	.82	1.71	2.19

The level-planted corn had more smut galls per 1,000 plants and a larger average daily weight increase of culm and of grain than the furrow-planted corn in all six comparisons. The averages show that there were 6 times as many smut galls per 1,000 plants, 1.3 times as much culm growth, and 1.3 times as much grain growth per day in the level-planted corn as in the furrow-planted corn. The furrow-

planted rows from which the plants were taken for these determinations contained a total of 226 plants, 11 (or 49 per 1,000) of which were smutted. Of the 208 plants in the level-planted rows 37 (or 178 per 1,000) were smutted.

The number of internodes and the average length, average circumference, and average weight of the individual internodes of 60 plants from furrow and from level planting are shown in Table 5. These are the same plants for which data are presented in Table 4. The data are arranged in the order of the internodes on the plant, internode No. 1 being nearest the ground.

TABLE 5.—*Effects of furrow and level planting on the morphology of the culms of mature corn plants of the F_1 cross 37×49, grown on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Internode (No. 1 being the first above the ground)	Number of plants having the stated internode—		Average length of internode on plants—		Furrow planted expressed as a percentage of the level planted	Average circumference of internode on plants—		Furrow planted expressed as a percentage of the level planted	Average weight of internode on plants—		Furrow planted expressed as a percentage of the level planted
	Furrow planted	Level planted	Furrow planted	Level planted		Furrow planted	Level planted		Furrow planted	Level planted	
18	48	58	17.8	15.8	113	2.2	2.3	96	1.1	1.3	85
17	54	59	16.8	14.4	117	2.4	2.7	89	1.3	1.4	98
16	59	59	15.4	12.9	119	2.8	3.2	88	1.4	1.6	88
15	60	59	14.7	12.4	119	3.2	3.9	82	1.6	1.9	84
14	60	60	13.8	12.4	111	4.0	5.3	75	2.0	2.7	74
13	60	60	13.4	13.4	100	5.6	6.6	85	2.8	3.8	81
12	60	60	15.1	14.3	106	6.3	7.1	89	3.9	4.8	88
11	60	60	16.7	15.1	111	6.8	7.7	88	5.1	5.8	88
10	60	60	17.3	15.0	115	7.1	8.1	88	6.0	6.7	90
9	60	60	16.7	15.5	108	7.3	8.5	86	6.6	7.6	87
8	60	60	15.9	16.1	99	7.7	9.0	86	7.2	9.1	79
7	60	60	15.3	16.7	92	7.9	9.6	82	7.7	10.6	73
6	60	60	15.2	16.5	92	8.4	10.1	83	8.6	11.8	73
5	60	60	14.4	15.6	92	8.9	10.5	85	9.0	12.5	72
4	60	60	13.2	14.2	93	9.2	11.0	84	9.2	12.7	72
3	60	60	11.5	12.5	92	9.5	11.4	83	8.9	12.8	70
2	60	60	9.4	10.2	92	9.7	11.5	84	8.4	12.2	69
1	60	60	6.4	5.9	108	9.5	11.0	86	6.8	8.4	81
Total or average			259.0	248.9	104	118.5	139.5	85	97.6	127.7	76

In addition to the basic weights and measurements, the data for the furrow-planted corn are expressed as percentages of those for the level-planted corn. The internodes of the furrow-planted corn were equal or more than equal in length to those of the level-planted corn, except in the case of internodes 2 to 8, which were distinctly shorter. The greater length of internode 1 of the furrow-planted corn agrees with the field observation that the seedling growth of the furrow-planted corn was better than that of the level-planted corn.

All of the internode circumferences of the furrow-planted corn were less than those of the level-planted corn, with no outstanding variations that can be considered a trend.

All of the internode weights of the furrow-planted corn were less than those of the level-planted corn. The relative weights of internodes 2 to 8 of the furrow-planted corn appear to be consistently

less than most of the others. Internodes 13 and 14 also are distinctly lighter than neighboring internodes. Since more ears were attached at the thirteenth node than at any other, it may be that the lighter weight was in some way associated with ear production. The culms of the furrow-planted corn as a whole were taller but slenderer and lighter in weight than those of the level-planted corn. All of the smut galls were at the second and third nodes of the furrow-planted corn. In the level-planted corn, two-thirds were at the four lower nodes and the other third was scattered upward to as high as the tenth node. The nodes that tended to have the most smut infections were in that portion of the plant where the internodes were most retarded in growth by the furrow planting. In these experiments the relation is obvious between smut infection and vigor as expressed in relatively rapid growth.

DIFFERENCES IN THE PROPORTION OF GROWING TISSUE

Table 6 shows the relation between the average daily weight increase and the percentage of water in 40 plants of each of the two crosses, 37×86 and 49×99 , at 73 days after planting and at silking time. The data are arranged under each planting method in the order of the air-dry weights of the plants, those for the 10 heaviest plants of each group being shown at the top. The plants are the same as those of Tables 2 and 3.

TABLE 6.—*Relation between average daily weight increase and percentage of water in the plants of two F_1 crosses at two stages of development, grown on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Group and stage of development	Cross 37×86				Cross 49×99			
	Furrow planted		Level planted		Furrow planted		Level planted	
	Average daily weight increase	Water content	Average daily weight increase	Water content	Average daily weight increase	Water content	Average daily weight increase	Water content
	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent
Heaviest 10-plant group:								
73 days after planting.....	0.96	88.0	1.47	90.5	1.03	89.6	1.45	89.6
At silking.....	2.83	83.7	4.20	82.4	3.24	82.7	4.17	83.0
Second heaviest 10-plant group:								
73 days after planting.....	.76	86.7	1.22	89.3	.83	88.4	1.13	89.6
At silking.....	2.03	83.8	3.55	82.3	2.42	81.4	3.50	82.7
Third heaviest 10-plant group:								
73 days after planting.....	.55	85.8	1.07	89.2	.56	87.3	.84	89.4
At silking.....	1.72	83.8	3.09	82.5	2.12	80.3	3.03	82.2
Fourth heaviest 10-plant group:								
73 days after planting.....	.37	85.4	.83	88.6	.22	86.0	.50	88.4
At silking.....	1.28	82.0	2.57	82.4	1.43	81.3	2.75	82.3

In all eight comparisons of plants, both at 73 days after planting and at silking, the average daily weight increase was greater when the corn was level planted than when it was furrow planted.

In seven out of eight comparisons the corn 73 days after planting contained a larger percentage of water when level planted than when furrow planted. Also, with one exception, the percentage of water content decreased with the average air-dry weight of plants within each cross under each cultural method. Although the differences were small, it is clearly apparent from the general trend that there

was a direct association between the average daily weight increase and the percentage of water in the corn 73 days after planting. There also was a direct association between the average daily weight increase and the relative number of smut infections (Tables 2, 3) and between the percentage of water in the corn at this age and the relative number of smut infections.

The percentage of water in the silking plants did not show consistent trends, ranging from 2 to 8.1 per cent less and averaging 5.8 per cent less than that in the plants 73 days after planting. All of the internodes and most of the leaves of the 73-day corn were growing, and those of the silking corn had nearly completed growth. The greater percentage of water in the 73-day corn was probably due to its larger proportion of growing tissue. The greater percentage of water in the level-planted corn as compared with that in the furrow-planted corn also suggests a larger proportion of growing tissue in the former. This growing tissue may have included leaves, leaf sheaths, culms, and potential ear shoots.

The ear shoots obviously were much more susceptible to smut than any of the other plant parts. Usually infected ear shoots observed early in the development of the smut gall were found to be clearly differentiated into husks, cobs, and, in a relatively few cases, kernels. The husks usually escaped attack, although in vigorously growing plants of selfed line 86 and in some of its crosses very young husks sometimes were smutted. Here the fungus apparently developed in or near the meristematic portions. Most of the infections causing smut galls on the ear shoots occurred in the cobs and particularly in the ovules. Quite commonly the galls grew from the ovules, without superficial modification of the surrounding tissues. During several years of observation, kernels that had developed under field conditions to a stage where they could be recognized as such have been found smutted in only three or four ears.

These observations indicate that one of the conditions necessary for infection of corn by smut is the presence of very young, possibly meristematic, tissue.

Rapidly growing corn would be expected to contain a larger proportion of young tissue than slowly growing corn. The differences in moisture content noted indicate agreement with this expectation. This may be one reason for the direct association between differences in vigor and proportion of smutted plants.

INHERENT DIFFERENCES OF BREEDING LINES

Table 7 shows the relation between vigor, expressed by relative growth weights, and the relative number of smut galls in each of seven selfed lines of corn and F_1 crosses between them.

The average daily weight increase of plants up to silking is based on 10 representative plants of a selfed line selected so as to be most nearly comparable with the representative plants of other lines. The data on smut infection in 1927 were taken on the rows sampled for determining the average daily weight increase up to silking. The data on smut in the selfed lines and their crosses in 1925 were taken in adjacent plots. They are all arranged in the order of average daily increases in weight of selfed-line plants up to silking.

TABLE 7.—*Relation between vigor, expressed by relative growth weights, and the relative number of smut galls in each of seven selfed lines of corn and F₁ crosses between them as grown on Arlington Experiment Farm, Rosslyn, Va., in 1925 and 1927*

No.	Selfed lines								Crosses						
	Days from planting to silking in 1927				Smut in 1927		Smut in 1925		Average weight of grain per plant from crosses of the 1 line with the other 6 of the experiment in 1925						
	Averages of silking plants in 1927				Total plants		Galls per 1,000 plants		Expressed as a percentage of that from line No. 54						
	Plant weight	Daily weight increase	Daily weight increase expressed as a percentage of that from line No. 54						Total plants	Actual	Expressed as a percentage of that from line No. 54	Relative average vigor (column 12 divided by column 5)	Smut galls per 1,000 plants, actual	Relative infection (column 14 divided by column 9)	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
	No.	Gm.	Gm.	P. ct.	No.	No.	No.	No.	No.	Gm.	P. ct.	P. ct.	No.	P. ct.	
37.	113	324	2 87	119.1	99	424	94	500	1,372	345	95.8	80.4	246	49.2	
86.	108	283	2 62	108.7	168	393	262	660	2,123	350	97.2	89.4	310	47.0	
9.	110	274	2 49	103.3	96	250	255	165	1,243	345	95.8	92.7	82	49.7	
54.	107	258	2 41	100.0	215	88	230	83	1,553	360	100.0	100.0	143	172.3	
99.	109	227	2 08	86.3	216	69	285	74	2,292	353	98.1	113.7	136	183.8	
49.	108	183	1 69	70.1	54	185	229	0	2,498	325	90.3	128.8	62	8	
79.	119	194	1 63	67.6	43	47	130	62	1,435	323	89.7	132.7	274	441.9	

The number of days from planting to silking for the lines did not differ widely, except in the case of line 79, which required 6 days more than the line with the next longest period and 12 days more than the line with the shortest period. The average dry weights of silking plants and the average daily weight increases of silking plants of the different lines ranked the same, except for line 79. The daily weight increases expressed as a percentage of that of line 54 (column 5) show a range in the rate of growth of 51.5 per cent. The numbers of smut galls per 1,000 plants for the selfed lines in 1927 (column 7) are in the same order as daily increase in plant weight with the exception of that for line 49. The relative numbers of smut galls in 1925 (column 9) also are in this order except for lines 86 and 79. In both years the number of smut galls per 1,000 plants in each of the three most rapidly growing lines, 37, 86, and 9, was greater than that in any of the other four lines. The data for the two years are in substantial agreement and show a direct association between smut infection and relative vigor as expressed by the average daily increase in plant weight up to silking.

Data also are presented in Table 7 on the average of crosses between each line and each of the six other lines in the experiment. The highest average yield of grain per plant from the crosses in 1925 was from those of line 54, which is the line with the midrate of plant growth up to silking. The yields are presented in column 12 as percentages of the average yield of the crosses of line 54. The crosses of the three lines with rates of growth greater than line 54 produced from 2.8 to 4.2 per cent less, and the crosses of the three lines with rates of growth less than line 54 produced from 1.9 to 10.3 per cent less grain per plant

than those of line 54. In other words, the relative average vigor of the crosses of lines 37, 86, and 9 was proportionately less and that of the crosses of lines 99, 49, and 79 was proportionately greater than that of their respective parents. This is shown by dividing the percentage of yield of the hybrids (column 12) by the percentage of daily weight increase of the parent line (column 5). The ratios (column 13) are inversely proportional to the average daily weight increase up to silking of the parent selfed lines (column 5), the tendency being consistent throughout the seven lines; that is, the vigor in the crosses above that of the parents (column 13) was inversely proportional to the vigor of the parent lines (column 5). The relative numbers of smutted plants in the crosses were substantially in this same order (column 15), indicating an association between smut infection and increased vigor due to hybridity.

Table 8 shows the effect on the yield of the F_1 crosses of selecting and crossing selfed lines of corn with different degrees of resistance to smut.

TABLE 8.—Effect on yield of F_1 crosses of selecting and crossing selfed lines of corn with different degrees of resistance to smut, as grown on the Arlington Experiment Farm, Rosslyn, Va., in 1925

Relative resistance to smut of the selfed parent lines	F_1 crosses	Smut galls per 1,000 plants in the parent lines of the crosses		Weight of grain per plant	
		Average of the parent lines in each cross	Average of the parental averages in the group	For each cross	Average of the crosses
		Number	Number	Grams	Grams
Both parents susceptible.....	37×86	580	442	336	352
	9×86	413		312	
	9×37	333		407	
	79×99	68		336	
Both parents resistant.....	49×99	37	45	322	311
	49×79	31		276	

The number of smut galls per 1,000 plants of the parent lines of the susceptible group is from 4.9 to 18.7 times as large as that of the resistant group. The average of all the parent lines of the susceptible group is 9.8 times as large as that of the resistant group. Although the susceptible parent lines were much more diseased than the resistant lines, only one of the crosses (9×86) from them was less productive than any one of the crosses from the resistant parents. One cross from susceptible parents equaled the yield of the best-yielding cross from resistant parents, and one was distinctly more productive than any other in the comparison. The crosses from the susceptible group as a whole averaged 13.2 per cent more grain per plant than those from the resistant group.

It has been shown⁷ that ears well protected by husks are less susceptible to smut. The ears of selfed lines 9 and 86 and the cross between them had relatively poor husk covering, and smut galls commonly developed on the protruding ear tips of this cross. Many of these smutted ears had noticeably shriveled kernels, whereas the unsmutted ears of this and the other crosses did not show this defect, although smut galls were present on other parts of the plants. Smut

⁷KYLE, C. H. RELATION OF HUSK COVERING TO SMUT OF CORN EARS. U. S. Dept. Agr. Tech. Bul. 120, 8 p., illus. 1929.

infection apparently caused a disproportionate loss of grain in this cross, which in turn reduced the superiority in grain production of the susceptible crosses as a group.

The resistant lines in this experiment were relatively low in vigor, and the crosses between these lines, as a whole, produced less grain than those from the relatively more vigorous susceptible lines.

SUMMARY AND CONCLUSIONS

Corn lines that had been selfed 6 to 11 years and F_1 crosses between such lines were studied for relative vigor and smut reaction. Vigor was measured by relative size or by weight of total plant growth produced per unit of time or of grain produced by plants requiring about the same time to mature.

In 1925 crosses between relatively resistant lines and between relatively susceptible lines all had more smut galls per 1,000 plants than their less vigorous parents.

In 1926 plants of the same crosses differing in vigor were compared in 21 F_1 crosses. The results show that the vigorous plants had more smutted nodes and more smutted ears than the less vigorous plants.

In 1927 listed and level-planted corn were compared. The listed corn grew more slowly than the level-planted corn. The culms were slenderer and lighter in weight when mature and had fewer smut galls both when the furrowed land was leveled (73 days after planting) and when the corn was mature. The furrow-planted corn also produced less grain than the level-planted corn. The nodes that tended to have the most smut infections were in that portion of the plant where the internodes were most retarded in growth by the furrow planting.

The percentage of water in immature corn plants was directly associated with growth rate and the relative number of smut galls.

The relative vigor and susceptibility of seven selfed lines of corn and of F_1 crosses between them were studied. The number of smut galls per 1,000 plants in each of the three most vigorous selfed lines was greater than that in any of the four other lines in 1925 and 1927. In general tendency the effect of crossing on the vigor and susceptibility of these corn lines was the same, relative changes having been produced in accordance with a similar mode of inheritance. Extreme resistance, obtained by selection, was found in selfed lines of relatively low vigor; crosses between these lines, as a whole, produced less grain than those from the susceptible lines which were relatively vigorous.

The data presented show a clearly defined tendency in the corn plant for vigor to be directly associated with the relative number of smutted potential ear shoots. This association holds when the differences in vigor are caused (1) by differences in the environment, (2) by plant variations within crosses, (3) by differences in heterosis between crosses and their selfed parents, and (4) by differences inherent in the different selfed lines. In selecting smut-resistant selfed lines it must be borne in mind that smut resistance in some cases may be due to lack of vigor. The use of strains having such low vigor may result in lower yields.

A COMPARATIVE STUDY OF THE CITRUS BLAST BACTERIUM AND SOME OTHER ALLIED ORGANISMS¹

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INTRODUCTION

A previous report (25)² has been made of the pathogenicity and characteristics of the three organisms, *Bacterium citriputale* C. O. Smith, *Bact. syringae* (Van Hall) E. F. S., and *Bact. cerasi* (Griffin). The last should not be confused with *Phytomonas cerasi* Wragg Sackett, a yellow viscid organism producing a disease of leaves and fruit of Wragg cherry (20). *Bact. citriputale* was described in connection with black pit and blast of Citrus, *Bact. syringae* in connection with blight of lilac, and *Bact. cerasi* in connection with gummosis of cherries, apricots, and plums. This paper is intended to show the general similarity and certain differences of these closely related organisms and to bring together certain other information regarding them.

HISTORY AND DISTRIBUTION

Lilac blight due to *Bacterium syringae* was first described (15, p. 141-146, 191-198) from the Netherlands. The cultural characteristics and successful inoculations on lilac and other hosts by Beijerinck were reported.

The disease was found to be of minor importance in the Netherlands, as indicated in a letter from C. J. J. van Hall in 1927. Its occurrence has been reported in Illinois by Bryan (5), who has redescribed the organism in accordance with recent standards. The same disease has also been listed (23) from California on lilacs grown at Julian, situated in the mountains and subject to cool, moist conditions during the winter and spring. This disease has also been found in Germany (26) and possibly also in England (14). Bryan (5), however, points out that the symptoms of the disease in England as given by Güssow (14) are unlike those of the disease reported from the Netherlands.

Bacterium cerasi was first described by Griffin (13) and later by Barss (3, 4) as causing a gumming of sweet cherry in Oregon and was later found by Barrett (2) in California, attacking other species of *Prunus*, as the plum and apricot. It appears to be similar to cherry gummosis of Germany (1).

Bacterium citriputale was first reported by Smith (22) as causing sunken pits on lemons. Coit (7) and Hodgson (16) described a new disease of the orange which they called citrus blast. Later Fawcett, Horne, and Camp (11, 12) found that the causal agent of citrus blast, for which Lee (17) had worked out the name *Bact. citrarefaciens*, was identical with *Bact. citriputale*. The black pit on the fruit

¹ Received for publication Mar. 20, 1930; issued August, 1930. Paper No. 214, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

² Reference is made by number (italics) to "Literature cited," p. 245.

and blast on leaves and twigs has been reported in Italy by Savastano (21) and by Fawcett (9) in Sicily and Italy. It has been also reported in West Australia by Carne (6), in South Australia and Victoria by Lewcock (18), and in Palestine by Reichert and Perlberger (19). A disease in South Africa with similar lesions on Citrus has been described by Doidge (8) in connection with a different organism. Smith (23) reported briefly a disease of the lilac (fig. 3, E; fig. 4, G) in California and a spot of the fruit of avocado from both of which was isolated a species of bacterium that in cultural characteristics

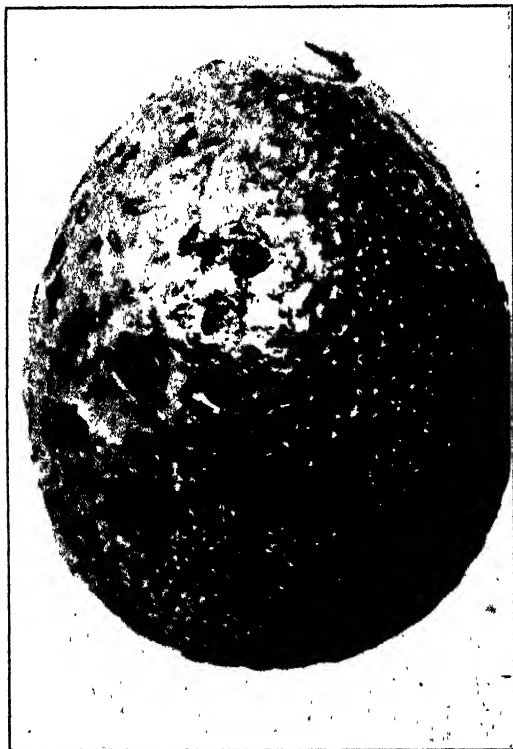


FIGURE 1.—Naturally occurring avocado blast on fruit of Queen avocado, *Persea americana*. Mature spots are circular to irregular in shape, brownish or black, and 3.5 to 6.5 mm. in diameter. They are superficial, being limited almost entirely to the rind

and pathogenicity closely resembles the citrus blast and black pit organism, *Bact. citriputeale*. This organism has been repeatedly isolated from black, water-soaked, or somewhat hardened tissue of avocado fruits. (Fig. 1.) It was also isolated from blackish depressed tissue surrounding the lenticels. It has been cultured from the Knight, Blackman, Taft, and Queen varieties of avocado, *Persea americana* Mill.

PATHOGENICITY ON DIFFERENT HOSTS

Van Hall (15) reported positive infections from inoculations with *Bacterium syringae* on lilac, *Populus nigra*, apple tree, pear tree, *Prunus mahaleb*, buckwheat, and *Atriplex hortensis*. Fawcett, Horne, and Camp (12) successfully infected with *Bact. citriputeale* two species of California oak, (*Quercus agrifolia* Née. and *Q. wislizenii* A. DC.

They also noted lesions on the latter oak that closely resembled those on Citrus. Although they failed to isolate the organism directly from these lesions on oak, they were able to obtain it indirectly by using these lesions as a source of inoculum on lemon fruits. Typical black pit lesions developed from which the organism was recovered. Bryan (5) has given illustrations of lesions on lilac leaf petioles by artificial inoculations with *Bact. citriputeale*. The same author produced black pit lesions on lemons by needle pricks with *Bact. syringae* of both the Netherlands and the Illinois strain. These strains, however, produced less effect on lemons than *Bact. citriputeale*. Compare size of lesions in temperature relations. (Table 1.)

SOURCE OF CULTURES

The cultures used in making artificial inoculations reported in the present paper were isolated from the following hosts: *Bacterium citriputeale* from lemon fruits and navel-orange twigs in Australia and California, and fruit of the avocado in California; *Bact. cerasi* from apricot and cherry twigs in California; *Bact. syringae* from lilac in California, and a culture isolated from lilacs from the Netherlands supplied by Mary K. Bryan of the United States Department of Agriculture.

ARTIFICIAL INOCULATIONS

Inoculations with pure cultures of these three organisms, *Bacterium cerasi*, *Bact. citriputeale*, and *Bact. syringae* into punctures produced definite lesions (fig. 2, A-V; fig. 3, A-T; fig. 4, A-I) in the following plants: *Carissa grandiflora* DC.; *Chalcas exotica* Millsp.; *Citrus aurantii* Linn.; *C. limonia* Osbeck; *C. sinensis* Osbeck; *Coprosma baueri* Endl.; *Fraxinus ornus* Linn. (*F. floribunda* S. P. 1. 47687); *Jasminum primulinum* Hemsl.; *Juglans regia* Linn.; *Lycopersicum esculentum* Mill.; *Malus sylvestris* Mill.; *Musa paradisiaca* Linn.; *Nerium oleander* Linn.; *Persea americana* Mill.; *Populus* sp.; *Prunus armeniaca* Linn.; *P. persica* Lieb. and Zucc.; *P. pumila* Linn.; *P. salicina* Lindl.; *Syringa vulgaris* Linn.; *S. japonica* Decne.; and *S. persica* var. *laciniata* Vahl.

In puncture inoculations on *Prunus armeniaca* definite lesions (fig. 2, P-R) were produced on the fruit, but only slight lesions and gum formation on the twigs, except with inoculations of *Bacterium cerasi* where a 20 to 30 mm. lesion developed. On the fruit, twigs, and leaves of *Persea americana* (avocado) (fig. 2, J-M; fig. 3, A-D; fig. 4, A-C) and Citrus species (orange and lemon) definite lesions (fig. 2, A-D; fig. 3, G-I) also developed from puncture inoculation. On the twigs of *Chalcas exotica* and on both twigs and leaves of *Coprosma baueri* (fig. 3, R-S; fig. 4, H-I), *Syringa vulgaris* (fig. 3, F), and *S. persica* var. *laciniata* typical lesions developed especially on young, rapidly growing tissue. (Fig. 4, D-E.) On twigs of *Fraxinus ornus* (*F. floribunda*) (fig. 3, M), *Populus* sp. (fig. 3, N-Q, and *Nerium oleander*, lesions readily developed on the newer tissue. Lesions also were formed on both fruit and twigs of *Malus sylvestris* (fig. 2, Q; fig. 3, J-L) and *Pyrus communis*, these being especially large on the fruit of *Pyrus communis*. (Fig. 2, E-I.) The lesions on the midvein of leaves of *Musa* were 10 to 20 mm. long and became dark colored. On the nuts of *Juglans regia* (fig. 2, T-V), the lesions that resulted from puncture inoculations were depressed and small (2 to 7 mm.).

Most of the lesions on the different hosts resulting from puncture inoculations developed in 10 to 14 days, showing depressed brownish to black areas usually 5 to 10 mm. long and about 3 to 4 mm. wide. The characteristics of lesions on different hosts were in general similar, but certain differences were noted; for example, on the twigs of *Persea americana* (avocado) and *Populus* a narrow, dark-colored streak frequently developed from the puncture inoculation and extended 20 to 30 mm. When examined microscopically, a brownish color was found in the cortex and xylem. The vascular part became involved from the original puncture and apparently carried the organism short distances. Fruits of *Lycopersicum esculentum* (tomato), inoculated a

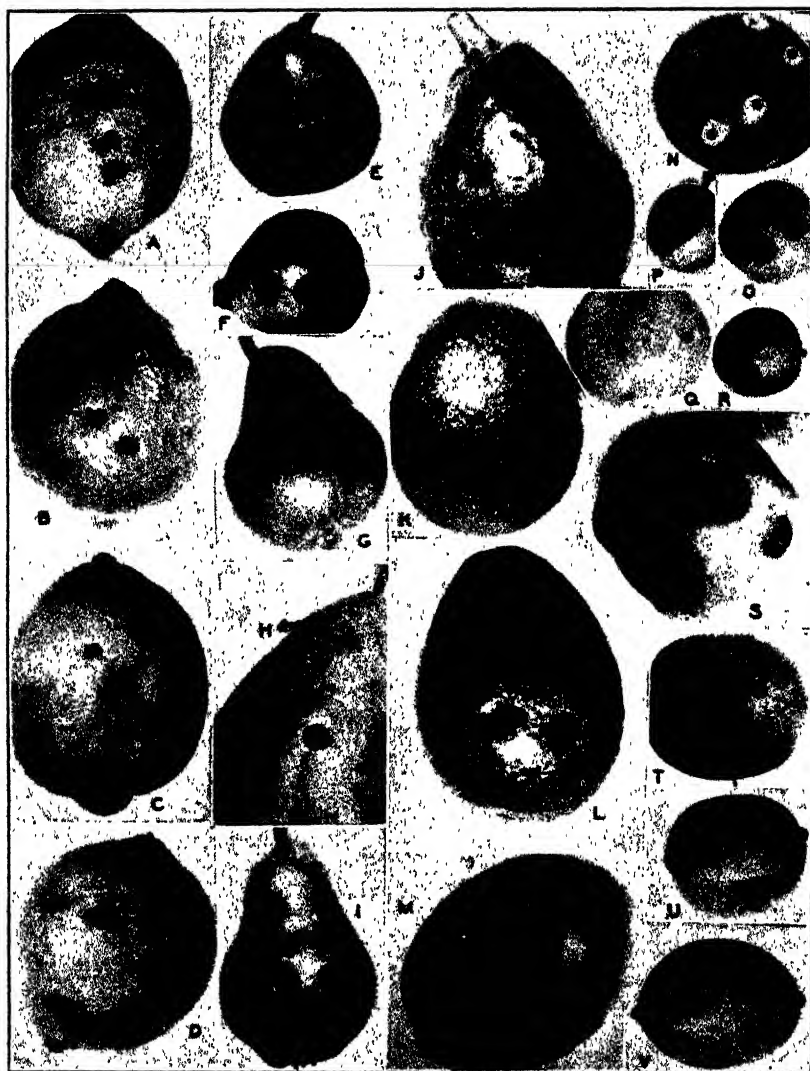


FIGURE 2.—Artificial puncture inoculations on fruits with citrus blast and other closely related organisms

A-D.—Inoculations on lemons: A, With apricot gummosis organism *Bacterium cerasi*; B, with avocado blast strain of *Bact. citripuleale*; C, with citrus blast organism, *Bact. citripuleale*, from orange twigs from California; D, with lilac blight, *Bact. syringae*, from the Netherlands.

E-I.—Puncture inoculations on pears: E, With apricot gummosis organism; F and G with avocado strain of citrus blast; H, with California strain of citrus blast from orange; I, with California lilac organism.

J-M.—Inoculation on avocados by puncture: J, Puebla avocado with the apricot gummosis organism; K, Puebla avocado with the avocado blast organism; L, Puebla avocado with the citrus blast organism from Australia; M, Ganter avocado with the Netherlands strain of lilac blight.

N.—Tomato inoculated with California citrus blast organism.

O-R.—Royal apricots inoculated by puncture: O, With lilac blight organism from Netherlands; P, with culture of apricot gummosis; R, with citrus blast from Australia.

S.—Peach inoculated by hypodermic injection with citrus blast culture.

T-V.—English walnuts, puncture inoculated: T, With apricot gummosis organism; U, with citrus blast from Australia; V, with the California lilac organism.

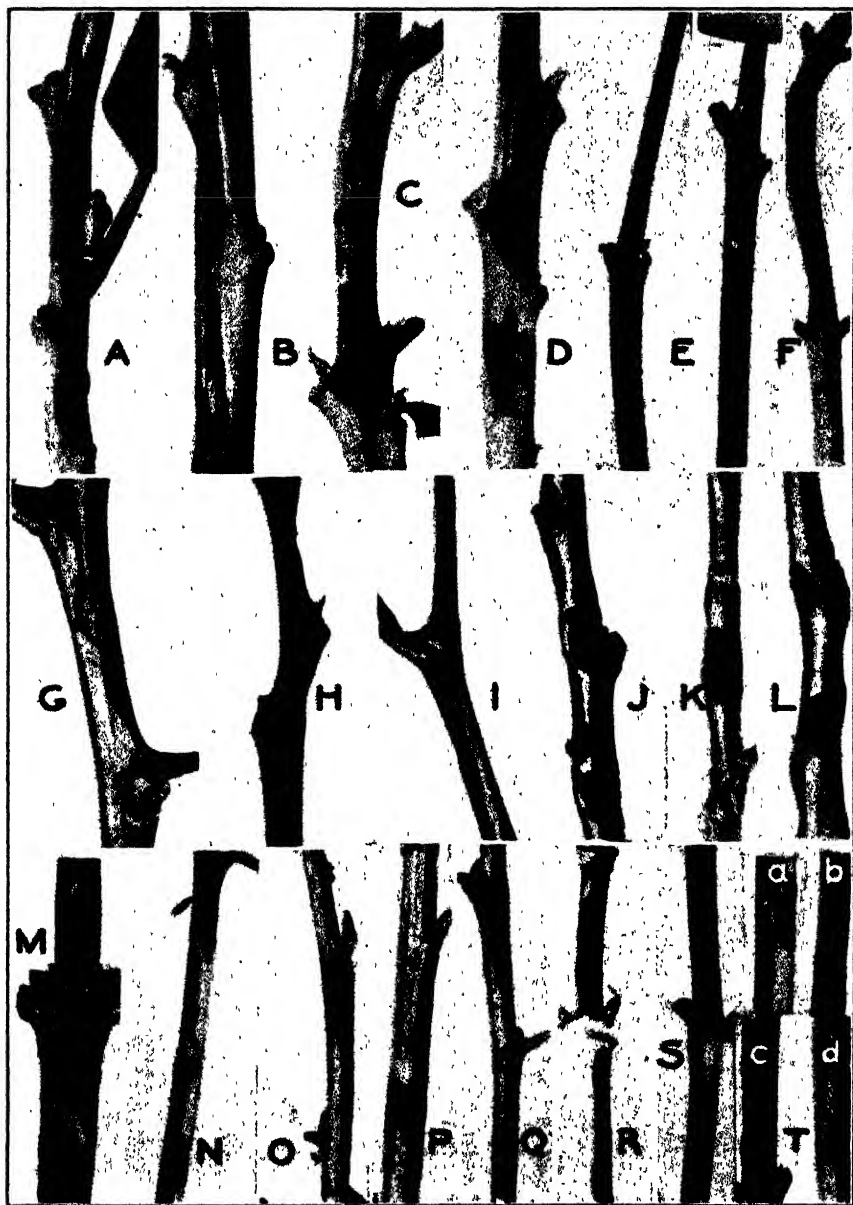


FIGURE 3.—Puncture inoculations on twigs of different hosts

A-D.—On shoot of avocado: A, With California lilac organism, *Bacterium syringae*; B, with apricot gummosis, *Bact. cerasi*; C, with avocado blast organism, *Bact. citriputeale*; D, with citrus blast, *Bact. citriputeale*, from Australia.

E.—Natural infection of lilac with *Bact. syringae*.

F.—Lilac, *Syringa vulgaris*, inoculated with avocado blast.

G-I.—Inoculation on citrus: G, Sour orange with culture of Netherlands lilac blight; H, grapefruit (pomelo) with avocado blast; I, sour orange with citrus blast from Australia.

J-L.—Inoculations on apple: J, With California lilac blight; K, with apricot gummosis; L, with citrus blast from Australia.

M.—*Frazinus ornus* inoculated with avocado blast.

N-Q.—Inoculations on *Populus* sp.: N, With citrus blast from California; O, with avocado blast; P, with apricot gummosis; Q, with California lilac blight.

R-S.—Inoculations on *Coprosma baueri*: R, With citrus blast from Australia; S, with California lilac organism.

T.—Inoculation of *Jasminum primulinum*: a, Lilac organism from Netherlands; b, California citrus blast; c, apricot gummosis; d, with avocado blast.

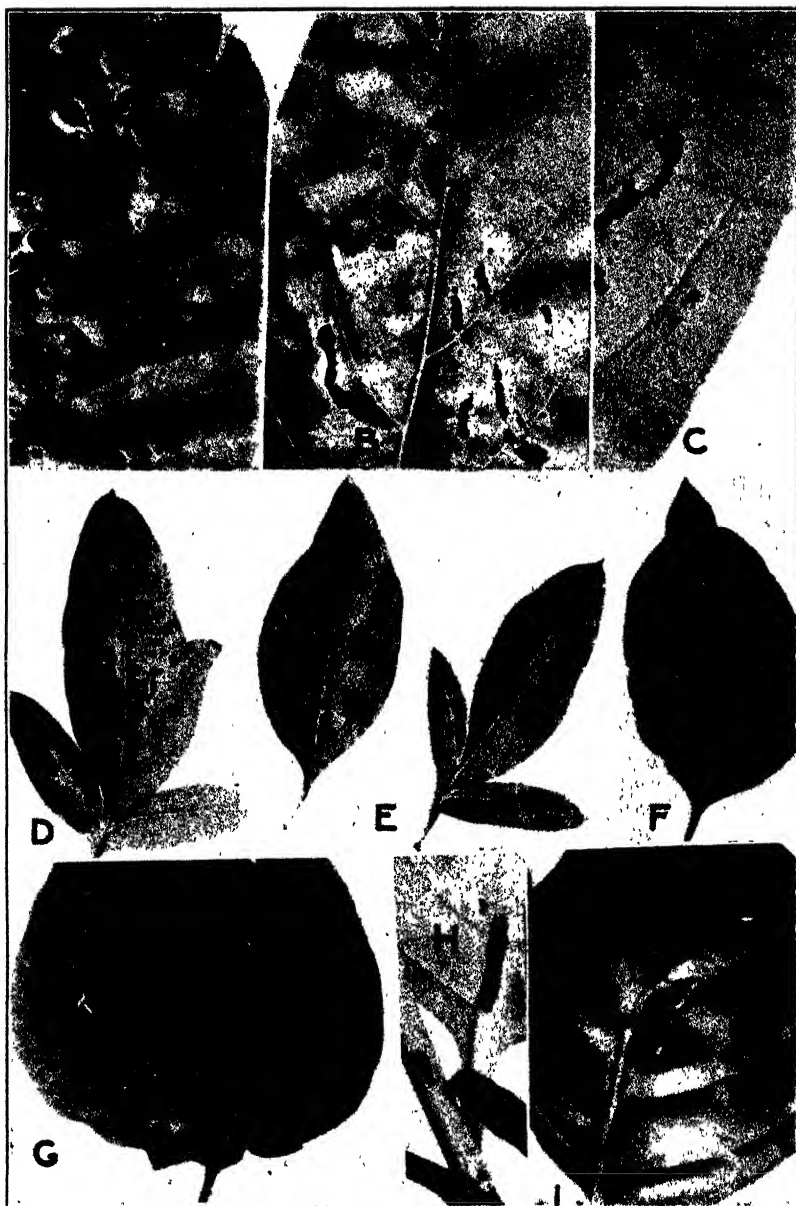


FIGURE 4.—Artificial inoculations on the leaves of different hosts

A-C.—Inoculation by brushing avocado leaves: A, With *Bacterium citriputeale* from citrus; B, with lilac organism, *Bact. syringae* (Netherlands strain); C, with avocado organism, *Bact. citriputeale*. D-F.—Inoculations on leaves of lilac: D, Puncture inoculations on *Syringa persica* var. *laciniata* with California lilac organism; E, puncture inoculations on *S. persica laciniata* with avocado strain of *Bact. citriputeale*; F, puncture inoculations with citrus blast organism from Australia.

G.—Natural infected lilac leaf from Julian, Calif., similar to those from which the lilac organism was isolated.

H-I.—Leaves of *Coprosma baueri* inoculated by punctures: H, With the avocado blast organism; I, with the citrus blast organism from Australia.

short time before they reddened, developed small black areas about 3 to 5 mm. in diameter, surrounded with a greenish zone that did not redden like normal tissue. (Fig. 2, N.) In the fruits of pear a blackened depressed area 5 to 20 mm. in diameter developed about the puncture. The small inoculated fruits fell prematurely, but the larger ones remained on the tree for some time. The infected area ceased to grow and a depression eventually developed.

Lesions were also produced on some hosts by brushing and atomizing without definite injuries. With the avocado, spots developed on the young tender leaves when brushed with the organism, especially when the brushing was done during a light rain. Under such circumstances the conditions for infection apparently were especially favorable. Atomized leaves of avocado that were still attached to the tree and kept under moist chambers have also shown positive lesions. These lesions on the leaves show either as a small spot a few millimeters in diameter with a yellow margin or as larger areas becoming brownish in color from which the dead tissue falls out, leaving holes in the leaves. (Fig. 4, A-C.) This effect has been found on avocado leaves where natural infection appears to have taken place during moist weather.

The succulent growth is more readily inoculated than the more woody tissue. It is probable that the tender succulent growth of a number of hosts besides those listed can, under favorable conditions, be successfully inoculated. The successful inoculation in most instances depended more upon the virulence of the culture than upon the source from which it was isolated. It was usually found that the organism when inoculated into the same host from which it was isolated, produced a larger and more spreading lesion. This was especially noticeable in the temperature experiments to be described later. (Table 1.) Larger lesions were caused by the citrus blast organism, especially in the albedo or white part of the rind than by the other organisms.

Artificial inoculations on apricot and cherry with these organisms under the climatic conditions of southern California did not produce the large lesions that girdle the tree trunk as reported on sweet cherry by Barss (4). Barss, however, secured these results only from the winter inoculations; his summer inoculations gave very much smaller lesions, never more than 15 to 25 mm. Puncture inoculations made during January in Riverside on sweet cherries with the more virulent cultures of *Bacterium citriputale* produced in three weeks definite lesions 20 to 30 by 10 to 15 mm. These are accompanied with much gum formation. These are comparable in size of lesion to those that have been produced on other hosts. The strain of *Bact. cerasi* from apricot and sweet cherry and of *Bact. syringa* from lilac when inoculated at room temperature by punctures in lemon fruits, developed typical black pit spots that were not distinguishable from those produced by *Bact. citriputale*. (Fig. 2, A-D.) However, differences in size of lesions at different temperatures should be noted.

OTHER PATHOGENES ON LEMON FRUITS

The characteristic positive lesions produced on lemons by these cultures from such widely different sources as lilac, avocado, Citrus, and Prunus (cherry and apricot) would suggest that possibly lemons might be readily inoculated with almost any species of bacteria patho-

genic to plant tissue. This, however, was not found to be true, for artificial inoculations with all other pathogenes tested gave negative results when placed in punctures in lemon fruit. The following were tested: *Bacillus amylovorus* Burrill, *Bacterium delphinii* E. F. S., *Bact. tabacum* Wolf and Foster, *Bact. tumefaciens* Smith and Townsend, and *Bact. juglandis* Pierce.

TEMPERATURE RELATIONS

It has been found in previous work (12; 10, p. 301, 449) that comparatively low temperatures (12.5° C. for lesions on citrus leaves in one inoculation by injection and 17° for black pit lesions on lemon fruits) are best for the formation of the largest lesions of citrus blast and black pit. In the following tests the largest spots occurred at 13.1° in 10 days for all stains except the avocado.

The comparative temperature tests here reported were made with light-green lemon fruits divided into seven lots of 60 fruits each, one lot for each temperature chamber. Fifty lemons of each lot were inoculated with the five strains of bacteria (10 lemons to each strain) which are indicated in Table 1. Ten other lemons were punctured and held as controls. Each fruit was inoculated with five punctures about 30 mm. apart distributed over one side. A suspension drop of the organism grown two days in Czapek's medium was placed on the surface and a puncture 2 mm. deep in the rind with a blunt needle was made through this drop. The fruits were then wrapped in citrus packing paper and placed in paper sacks at the various temperatures indicated. Table 1 shows the average size of spots at different temperatures and at different times. Each average is made up of five spots on each of 10 lemons, making 50 individual punctures for each average.

TABLE 1.—Average diameter of spots produced by inoculations on lemon fruits with five different strains of the citrus blast organism, when fruits were held thereafter at seven different temperatures

Strain and source of inoculum	Period kept	Diameter of spots when fruits were kept at temperatures indicated						
		°						
		5.8° C.	13.1° C.	17.4° C.	21.7° C.	26.5° C.	29.1° C.	31.5° C.
	Days	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
Citrus, California.....	3	0	1.8	5.3	5.4	5.5	3.6	2.8
	6	0	5.8	6.9	5.2	5.3	3.5	2.9
	10	1.7	8.1	5.6	4.5	5.3	3.8	3.0
Citrus, South Australia.....	3	0	1.9	5.3	5.3	4.0	2.8	1.9
	6	0	6.9	6.2	4.8	3.7	2.8	2.8
	10	3.0	8.0	6.2	4.8	3.4	2.9	3.6
Lilac, Europe.....	3	0	.8	2.9	4.3	2.9	1.1	1.6
	6	0	5.8	4.0	4.0	2.5	1.0	.9
	10	3.6	6.3	4.0	3.4	2.4	1.4	1.1
Apricot, California.....	3	0	.1	1.7	1.7	2.1	1.2	.8
	6	0	3.9	3.1	1.7	1.6	.9	.7
	10	3.5	4.0	3.1	1.7	1.7	1.2	1.1
Avocado, California.....	3	0	.9	4.0	5.3	3.7	1.4	.8
	6	0	4.6	5.4	5.4	3.1	2.0	.9
	10	3.4	5.0	5.4	5.4	3.1	2.2	.9

Reference to Table 1 shows that with the exception of the avocado strain the lesions 10 days after inoculation attain a maximum size at 13.1° C. and gradually decrease in size above this temperature. With the exception of the avocado strain, the largest spots at each temperature are produced by the two citrus strains. The sizes of

spots due to the two strains from California and the one from Australia coincide with each other fairly well. The sizes of the spots due to the European lilac and apricot strains decrease very rapidly at the medium and higher temperatures, being small and insignificant at temperatures at which the spots from the citrus strains are much larger. The apricot strain appears from these tests to be the least adapted for producing spots on lemon fruits. The spots due to the avocado strain reach their maximum size at a somewhat higher temperature, but they also decrease in size at the higher temperatures more rapidly than the spots caused by the citrus strains.

Experiments to determine the influence of temperature on the rate of enlargement of colonies of the different strains of bacteria were also carried out as follows: Glucose-potato agar was poured into Petri dishes and allowed to solidify. A platinum needle was dipped into a suspension of bacteria from a 3-day-old culture in Czapek's medium and the surface of the agar medium touched with the end of this needle. One inoculation for each strain in each of a set of four Petri dishes was made in each of the temperature chambers. Each Petri dish therefore contained one inoculation of each of the strains indicated in Table 2, and could be compared side by side.

TABLE 2.—Diameter of colonies of five different strains of bacterium grown at seven different temperatures

Strain and source of inoculum	Period of growth	Diameter of spots when growth occurred at temperatures indicated						
		5.8° C.	13.1° C.	17.4° C.	21.7° C.	26.5° C.	29.1° C.	31.5° C.
		Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
Citrus, California	Days							
	3	0	2.5*(2)	3.5	7.0(1)	7.5	7.3	4.8
Citrus, South Australia	5	0	7.5(2)	6.5	12.0	8.0	8.8	7.0
	3	0	2.3	3.5	22.0(1)	7.8	8.8	5.8
Lilac, Europe	5	0	7.5(2)	5.3	22.0	9.3(3)	10.3	8.0
	3	0	2.0	5.3	7.5(2)	12.5	10.0	4.0
Apricot, California	5	.3	3.0(2)	9.5	11.0	18.3	17.5	8.3
	3	0	2.5(2)	4.0	6.5(2)	5.5	7.8	7.0
Avocado, California	5	.5	4.5	8.8	22.0(2)	7.0	8.8	9.3
	3	0	3.5(2)	3.0	5.0(1)	5.7	7.3	3.5
	5	.3	5.0	5.0	9.0	8.0	8.5	8.3

* Numbers in parenthesis indicate the number of colonies, when less than 4, available for obtaining the average.

Considerable variation in different Petri dishes in the same chamber occurred in some cases, but the results as a whole are of interest. The temperatures at which the largest colonies were formed were, as a rule, higher than that at which the largest spots formed on lemon fruits. There seems to be no definite relation between the growth on glucose-potato agar and the rate of enlargement of spots on the fruit.

REISOLATIONS

These organisms have been frequently reisolated from the artificial inoculations and then reinoculated into lemon fruits. These results are summarized in Table 3. Dilution plates from a blackened streak on an avocado stem inoculated with the Australian citrus blast organism showed numerous colonies. The same was true for spots produced artificially on avocado leaves by brushing on them the

European lilac, citrus, and avocado blast organisms. In some positive inoculations on *Coprosma baueri* with the citrus-blast organism, the causal organism was reisolated from drops of rain water that stood on the leaves in the neighborhood of the positive lesion.

TABLE 3.—Reisolation of organisms studied

Host from which reisolation was made	Strain and source of the original inoculum	Result of inoculations with reisolated cultures into lemon fruits	
		Tests	Size of lesions produced
		Number	Mm.
Avocado stems.....	Lilac, California.....	10	3-5
Do.....	Avocado.....	10	5-10
Do.....	Orange.....	6	(*)
Avocado fruits.....	Lilac, California.....	9	5-10
Avocado leaf.....	Avocado.....	9	(*)
Do.....	Orange.....	8	3-8
Avocado stem.....	Citrus, Australia.....	10	2-5
Avocado leaf.....	Lilac, Europe.....	15	2-10
Carissa fruit.....	Avocado, California.....	8	3-4
Do.....	Lilac, California.....	8	3-4
<i>Coprosma baueri</i> stem.....	Orange.....	10	2-10
Do.....	Apricot, California.....	10	2-10
<i>Fraxinus ornus</i>	Lilac, California.....	6	2-5
Lemon fruit.....	do.....	5	2-3
Do.....	Lilac, Europe.....	4	2-4
Do.....	Avocado, California.....	3	3-5
Do.....	Apricot, California.....	10	2-8
<i>Prunus pumila</i> stem.....	Lilac, California.....	16	3-10
Do.....	Citrus.....	10	3-10
Do.....	Apricot, California.....	15	3-10
Plum fruit.....	Lilac, California.....	8	3-4
Peach fruit.....	Avocado, California.....	8	3-4
Apple stem.....	do.....	6	3-8
Do.....	Lilac, California.....	3	2-4
Tomato fruit.....	do.....	7	3-5
Do.....	Avocado, California.....	10	2-10

* Typical.

MORPHOLOGY AND OTHER CHARACTERISTICS

The characteristics as given for *Bacterium citriputeale* (15), *Bact. citrarefaciens* (17), *Bact. syringae* (5, 15), and *Bact. cerasi* (3, 13) agree in most respects, although sometimes the accounts are too meager for comparison. Certain differences in the descriptions, however, exist and these will be considered here. In the morphology of these organisms all accounts describe the organisms as motile by 1 to 3 flagella. Bryan (5) notes a small capsule for the lilac organism. Lee (17) reports no capsule for the citrus blast organisms, and no mention is made regarding the presence of capsule in the description of *Bact. cerasi*.

The organisms from Citrus, Prunus, lilac, and avocado were stained with Ribbert's dahlia stain, and all showed a very small capsule similar to that of the lilac organism.

The sizes of the rods as reported in literature are as follows:

Bacterium citriputeale (Smith) (22) 2-4 x 0.5-1 μ .

Bacterium citrarefaciens (Lee) (17) 1.2-3 x 0.3-0.9 μ (most common size 1.8 x 0.61 μ).

Bacterium syringae (Van Hall) (15) 1.6-3.2 x 0.2-0.4 μ .

Bacterium syringae (Bryan) (5) 1.2-1.8 x 0.6 μ .

Bacterium cerasi (Griffin) (13) 1.5-2.3 x 0.5-1.84 μ (majority 1.84 x 0.84 μ).

Lee reports for the citrus blast organism that a definite coagulum forms in milk and peptonizes slowly. Fawcett, Horne, Camp, and Smith observed no coagulum, but a rapid peptonizing or clearing of the milk. Griffin, as reported by Barss (3, p. 210), observed an alkaline reaction and prompt reduction of litmus milk. Bryan describes for the lilac organism a narrow 3 to 5 mm. layer of whey at the top of milk culture, but no further evidence of coagulation. Peptonization takes place almost at once, proceeding downward in zones. All authors agree that each of the three organisms causes the milk to become alkaline.

Further observations have been made on the cultures of these three organisms in litmus milk. At first a narrow layer of whey is often produced, followed by a rapid clearing and bluing of the litmus, thus often forming zones of colors. This is in agreement with the observation of Bryan. It sometimes happens that the clearing takes place without the layer of whey being visibly produced, or the reaction is so obscured that it is uncertain whether a coagulum of milk has taken place. A solid curd is never separated from the whey. Both Lee and Smith report that indol is produced for citrus blast. Bryan and Van Hall report no indol for the lilac organism.

The tests by Lee and by Smith were with the sodium-nitrate sulphuric-acid test. This test is now regarded as untrustworthy and is replaced by more accurate methods. A test for indol production by these different organisms was made during the present studies using the vanillin method. All cultures showed an absence of indol after an incubation of 10 days in Dunham solution.

STUDY OF SMOOTH AND ROUGH FORMS OF THE CITRUS BLAST ORGANISM

Cultures of the citrus blast bacterium when grown continuously on glucose-potato agar for a year or more may change from the characteristic smooth growth to one much more wrinkled. The virulence of the rough organism is also lessened, and its ability to liquefy gelatin is decreased or is absent. Some observations also with a culture isolated from avocado were made. There were several tubes of this isolation, some of which showed the smooth typical growth, while others showed the wrinkled growth. One tube of the wrinkled type was selected and a tube of Dunham medium inoculated. In dilution plates poured from it both the smooth, piled-up colonies, and the flatter more wrinkled ones were observed in the same plates. Subcultures were grown from each kind separately, and inoculations were made from them into lemon fruits. The type producing smooth growth gave typical black pit lesions. The type producing rough, wrinkled growth was found to be only slightly pathogenic and produced small lesions

FERMENTATION OF SUGAR

No gas was produced. Acid reaction showed in three days with 2 per cent saccharose, dextrose, galactose, levulose, and glycerin in Dunham's and Czapek's media. No acid reaction showed with lactose or maltose. An alkaline reaction in peptone could be detected after about six days, with brome cresol purple and phenol red as indicators. Lee (17) did not detect the formation of acid with litmus,

the more sensitive indicators not being commonly available then for laboratory use.

With the cherry gummosis organism Griffin, as recorded by Barss (3, p. 210), reports no gas production with sugars, but acid production with dextrose, saccharose, lactose, maltose, glycerin, and mannite. His observation that lactose and maltose produce acid is not in agreement with the reaction of the present writers' strain of *Bacterium cerasi* from apricot, which reacted like the citrus blast organism and produced no acid with lactose and maltose.

The senior author (23) reported that *Bacterium citriputeale*, the cause of citrus blast and black pit, was also responsible for a new lilac blight in California. He stated that "the results thus far indicate that the citrus blast, the avocado blemish and the California lilac disease are closely related and probably caused by the same organism." Stimulated by this report, Bryan (5) made a comparative study of strains of *Bact. syringae* isolated from lilacs in Illinois and in the Netherlands and a freshly isolated strain of *Bact. citriputeale* received from Fawcett in California. Bryan states that—

parallel cultures proved the latter to be identical with *Bact. syringae* except in colony characteristics on agar, and the differences in these were no more striking than the differences between the Netherlands and Illinois strains.

DISCUSSION AND CONCLUSIONS

The three bacterial organisms: *Bacterium syringae*, the blight of Syringa, *Bact. cerasi*, the gummosis of Prunus, and *Bact. citriputeale*, the blast of citrus, and *Persea americana* (avocado) have been studied in parallel cultures, and a close agreement in cultural and biochemical characters has been observed. These seem to form a group of closely related organisms.

From the comparisons in the present study it seems probable that the lilac blight of California is identical with that of Illinois and of the Netherlands, which is caused by *Bacterium syringae*, recently re-described by Bryan (5).

The organisms from Citrus and avocado, and apricot may be considered as having similar general cultural characteristics but show some differences in their pathogenicity which suggest that they may not belong to a common species.

The pathogenicity of the organisms listed is characterized by their forming small lesions from artificial inoculation on numerous hosts as follows: Fruits, leaves, and stems of *Persea americana* (avocado); leaves and stems of *Syringae vulgaris*, *S. persica*, var. *lanciniata*; stems of *Coprosoma baueri*, Citrus, Populus, and *Fracinus ornus*; and the fruits of *Citrus limonia* (lemon) and *Prunus armeniaca* (apricot). The small lesions and local gumming on apricot stems took place with all organisms except *Bacterium cerasi* where dark colored lesions 20 to 30 or more millimeters long were formed on the succulent twigs.

Temperature reactions of these four organisms when inoculated into lemon fruits cause lesions that agree well in size and other characters at 17° to 20° C., but at 29° to 31° the citrus blast group is sharply differentiated from the others by the larger size of lesion. (Table 1.)

There is a general agreement in cultural characteristics of these three organisms which would suggest that they might belong to a

common species. This, however, in itself is not sufficient, because bacteria of different species frequently have similar cultural characteristics and yet are not considered identical. The pathogenicity with these organisms is shown in so many different hosts, where puncture inoculated, that it loses in a measure its value as a distinguishing character, yet inoculations frequently suggest that the organisms are not entirely alike.

With our present knowledge of this group, the authors would hesitate to place these three organisms, *Bacterium syringae*, *Bact. cerasi* and *Bact. citriputeale*, in a single species. Should this ever be done, *Bact. syringae* from its priority would be the correct scientific name.

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SOME PHYSIOLOGICAL STUDIES OF PHYTOMONAS CITRI¹

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INTRODUCTION

Phytophthora citri (Hasse) Com. S.A.B., the organism causing citrus canker, which was isolated and described in 1915 by Hasse (12)² has been studied by many workers in an effort to discover some practical means of controlling the disease. How the organism is carried over from one outbreak of the disease to the next is not understood. The origin of some fresh outbreaks has been explained, but the source of most of those which have occurred in Florida since 1919 has not been determined. The causal organism has been found to produce neither spores nor protective cysts which would enable it to withstand long periods of adverse conditions. In view of these facts it seemed necessary to find under what conditions it can live in a more or less active state of growth.

In considering the possibilities of hold-over, it may be noted that bacteria are tolerant of many varied environmental conditions. They may live for a considerable length of time in the air, in water, in the soil, on tools, in decayed matter, on host plants, and on animals. Experiments with plant pathogenic bacteria have shown that ordinarily they are not able to live in the intestinal tract of animals. Consequently, for the present, this source of hold-over is not considered. Because experiments have shown that *Phytophthora citri* can not withstand long periods of drying, it was considered unlikely that the organism could hold over on tools and similar appliances or otherwise in direct contact with the air. This leaves the soil, water, decaying vegetable matter, and the various host plants as possible centers of infection. The first three, representing nonliving carriers, may be grouped together, and in these experiments have been studied under the category of various soil types. As the rôle of plant hosts in carrying over the organism will be treated in a later paper, it is not considered here. The present paper sets forth the results of studies of the effect of various temperatures upon the development and activities of *P. citri* growing in potato broth as shown by change in population. The rate-of-growth curves were calculated, and the change in hydrogen-ion concentration and starch content of the media were determined at frequent intervals during the incubation period. One of these experiments was started in October, 1927, and the other in November, 1928.

¹ Received for publication Jan. 23, 1930; issued August, 1930. That part of this paper which deals with the activities of *P. citri* in potato broth at various temperatures is a portion of a thesis submitted to the graduate committee of the University of Florida in partial fulfillment of the requirements for the degree of master of science, conferred in July, 1929. This work was done under the direction of the department of botany and bacteriology of the University of Florida.

² Reference is made by number (italic) to "Literature cited." p. 257.

REVIEW OF LITERATURE

During the early part of the citrus-canker eradication campaign it was generally assumed that *Phytophthora citri* could live in the soil for long periods. In 1915 Stevens (21) found that the organisms lived for a long time in sterile soil, and concluded that field soil was a source of danger. Stirling (23) in 1915 reported that in every instance where infected trees were destroyed and the ground was not immediately burned over, the shoots which grew up from the old stumps became infected. Wolf (25), in 1916, reported that the citrus-canker organisms remained active in the soil, as was indicated by the reinfection of sprouts. Jehle (14), in 1916, found that the organisms were able to live in the soil for a long time. He reported that red clay and glade soil inoculated with *P. citri* caused reinfection after 47 days. In 1920 Doidge (9) stated that sprouts coming from the soil were sometimes infected by bacteria in the soil. In 1929 she (10) reported that she was able to obtain viable canker organisms from the soil one year after infected trees had been destroyed and the citrus canker eliminated. In an experiment with reinoculated sterilized and unsterilized soil, she found that *P. citri* lived for 22 days in each. Judging from these results and observations, it is certain that the soil should be looked upon as a possible source of hold-over for citrus canker.

On the other hand, statements are to be found which show that under certain conditions the citrus-canker organisms die very soon in field soil. In some of his later experiments Stevens (22) found that soil samples taken from beneath infected trees usually gave negative results when inoculated into susceptible hosts. Fulton (11, p. 223), working with many types of soil, reported in 1920 that there was a rapid decline of *Phytophthora citri* in all soils tested, and said, "All these considerations suggest that agricultural soils probably can not long retain a dangerous possibility of disseminating the citrus-canker organism." Lee (16) found that the organism disappeared from unsterilized soil but lived and multiplied in sterile soil. In 1921 he (17) reported that in his clean-up procedure in the Philippine Islands he omitted the spraying of the soil to kill the *P. citri* organism because he considered such a precaution useless. Some of the reported decline of *P. citri* in the soil may be explained in the light of Hino's work (13) in 1926. He found that in experiments carried on with *P. citri* and other plant pathogens all of these organisms were destroyed when protozoa were added to them in sufficient numbers.

CULTURAL CHARACTERS

The strain of *Phytophthora citri* studied here agrees in all of its general cultural characters and biochemical reactions with those described by Hasse (12), Jehle (15), Doidge (8), and others, except as noted for the thermal death point.

Gas was produced from galactose, fructose, mannite, glucose, lactose, maltose, sucrose, asparagin, and amygdalin.

The usual bacteriological liquid media were tried, such as nutrient broth, litmus milk, Russell's double sugar, Uschinsky's solution, and others. Of these, potato broth containing 1 per cent dextrose and 1 per cent peptone was found to give the best growth.

Potato agar, prepared by adding agar to the broth mentioned above, was used for the isolations. On this medium the surface colonies appeared pale yellow by reflected light and bluish by transmitted light. They were circular in outline, entire, raised, smooth, glistening, and viscid. Submerged colonies were elliptic in shape.

One part of hydrogen peroxide to 2 parts of water killed the organism in one-half minute. It was unable to live for 1 day in 5 per cent bergamot, lemon, lime, or bitter-orange oils. As shown by inoculation tests into grapefruit leaves, the organism was able to live for 74 days in beef broth, 216 days in distilled water, 202 days in sterilized tap water, 165 days on sterile pine shavings, 290 days on potato agar, 120 days in potato broth, and 200 days in sterile sandy soil. No cultures of the organism have been obtained from herbarium specimens of the disease on grapefruit leaves which had been stored for 30 days.

The pH range at which *Phytophthora citri* can live in culture media has been found to be 4.5 to 9.5, with an optimum reaction of 6 to 8.6.

The thermal death point has been reported by Peltier (19) as being 49° to 52° C. and by Wolf (25) as 65°. In the present experiments in which tests were run with 45 and 4 day old cultures, growing in potato broth, the thermal death point in each instance was found to be 48° for five minutes. The tests were made in thin-walled sealed ampules at temperature intervals of 2° from 40° to 60° C. The difference in results obtained by the writer and those reported by other workers may be due to the difference in the method of testing or to the nutrient medium used, which, according to Williams (24), working with *Bacillus subtilis*, caused a change in the resistance of the organism to heat.

EFFECT OF TEMPERATURE ON RATE OF GROWTH OF PHYTHOMONAS CITRI IN POTATO BROTH

According to Bergey (2), the optimum temperature for the growth of *Phytophthora citri* is 25° C. Peltier and Neal (20) reported the overwintering of the citrus-canker organism where the temperature had fallen as low as -9°. Peltier (19) found that the minimum temperature for growth was 5°, the optimum from 20° to 30°, and the maximum from 33° to 35°. Doidge (9) stated, "It grows well at 30° C., rather slowly at 25° C., and very slow progress is made at 20° C."

Concerning the temperature at which infection will take place, Peltier (19) found that 20° C. was the minimum, 20° to 30° the optimum, and 35° the maximum. Based on experiments conducted at the Alabama experiment station, the United States Department of Agriculture (1) reported that citrus trees in any region having a mean temperature for the year of 20° or less will not become severely infected with citrus canker; however, in regions averaging 27° or higher one should expect severe attacks from infection. No maximum temperature was given at which infection would occur. For the most part, these findings were confirmed by tests made at the Florida Agricultural Experiment Station with grapefruit seedlings which were grown on nutrient agar in sterile test tubes and held

at various temperatures. The optimum temperature range appeared to be from 25° to 30° , as lesions developed in from 4 to 6 days at these temperatures. Plants which were held at 15° following inoculation had lesions on them at the end of 64 days. Other plants, held at 15° for 30 days without developing lesions, when transferred to a temperature of 25° developed lesions in 4 days. No information regarding infection at 35° was possible, because the plants inoculated and held at this temperature continuously for 5 days were killed by the high temperatures.

The apparatus used to control the temperature chambers for incubation was very similar to the one described by Camp and Walker (6), which was a modification of the soil-tank apparatus used by Livingston and Fawcett (18). Test cultures instead of soil were placed inside the pots, following which the pots were stoppered with large corks.

The type of flask used for growing the cultures was suggested by A. F. Camp of this station. (Fig. 1.) A bent glass tube which reached to the bottom of the flask opened into the test tube, which was suspended from the 2-hole stopper. Except for being loosely plugged with cotton, the L glass tubing was open to the air. By applying suction to this open end the liquid could be drawn from the flask into the test tube. This permitted the removal of samples of the culture without danger of contamination. By flaming the rubber stopper and empty sterile test tubes, an exchange of tubes, empty for full, could easily and quickly

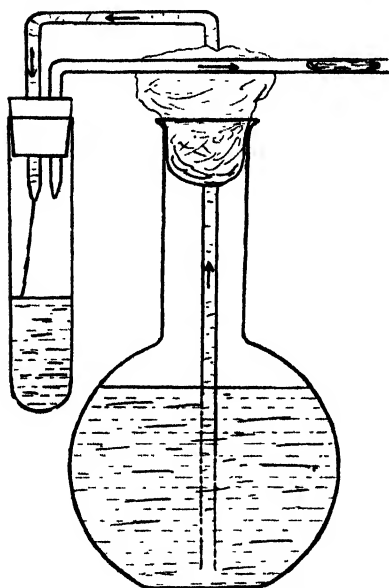


FIGURE 1.—Culture flask arranged with side-delivery apparatus for removing cultures without danger of contamination

be accomplished. These test flasks were prepared with the desired amount of liquid medium and the whole apparatus was sterilized at 15 pounds pressure for 15 minutes.

The medium used was potato broth, which was made according to the following formula: Extract from 300 gm. of potatoes cooked in a double boiler; dextrose, 10 gm.; peptone, 10 gm.; and distilled water to make 1,000 c. c.

The culture of *Phytophthora citri* used in the first experiment was isolated from grapefruit leaves, and that used in the second experiment was a transfer of the same culture, whose vigor had been maintained by frequent transfers. All of the stock cultures in these experiments were incubated at a temperature of 28° C. They were plated out at regular intervals, and frequent inoculations were made into grapefruit plants to prove their identity.

In the first experiment the temperatures in the incubation compartments were 11° , 16° , 20° , 24° , and 28° C. In the second experiment

six compartments were used and temperatures employed were $11\frac{1}{2}^{\circ}$, $17\frac{1}{2}^{\circ}$, 21° , 25° , $29\frac{1}{2}^{\circ}$, and $34\frac{1}{2}^{\circ}$.

Observations during the first experiment were made every 24 hours with the exception of a few days on which no data were taken. Curves showing the rate of growth in this experiment are presented in Figure 2. It is evident from these curves that the rate of multiplication during the first few days was very rapid. In order to determine more definitely what occurred during those first days, the observations in the second experiment were made every 12 hours for the first 16 days and every 24 or 48 hours thereafter.

When the test cultures were seeded an attempt was made to inoculate each flask with the same number of bacteria per cubic centimeter of medium. The stock cultures for each experiment were grown in a portion of the same lot of potato broth that was used for the tests in that experiment. When the first experiment was started the number of bacteria per cubic centimeter of the stock culture was determined by direct microscopic count, and enough of the stock culture was used to give the test cultures approximately 3,000 bacteria per cubic centimeter. In the second experiment enough inoculum was used to give each test culture approximately 30 bacteria per cubic centimeter. During the first experiment one culture was grown at each of the temperatures employed, but in the second the flasks were run in duplicate.

Whenever a sample was desired from a test culture the operation was performed as rapidly as possible so as not to change the temperature of the culture more than was necessary. If a stained slide showed no contamination, the sample was used to determine its population, its relative starch content, and its pH value.

The dilution and plating-out method of counting bacteria was found to be very unsatisfactory; consequently, in these experiments the populations were counted by the direct microscopic method, as described by Breed and Brew (4). It was necessary to modify the procedure when the population became too dense to count without diluting. Since *Phytomonas citri* is a slime producer, distilled water was not a good diluting medium. After many reagents had been tried, a solution of 10 per cent acetic acid was found to give the best results. To overcome the tendency of acetic acid to form droplets, the slides were placed in a strong solution of potassium hydroxide, blotted but not wiped or flamed, and then ruled, after which the desired dilution was spread and the film dried. This gave a very good distribution of bacteria and did not interfere with the staining process.

An ocular micrometer disk with ruled squares on it was used for measuring the fields of bacteria to be counted. The logarithmic values of the average of 50 fields were recorded as the populations of the samples.

The upper set of graphs in Figure 2 shows the results of plotting time against the logarithmic values of population and gives the rate-of-growth curves for the first experiment. An examination of these curves shows that the one having the greatest slope, or where growth was fastest, is for 28° C. In other words, the highest temperature permitted the most rapid increase in numbers. At this temperature, however, the bacteria were dead at the end of 40 days.

Tests made with this culture showed that death was due to an insufficient supply of food and not to any bacteriophage or other lytic principle.

The bacteria held at the other temperatures were still alive on the fifty-seventh day. The culture which was held at 11° C. became contaminated after forty-nine days and was discarded. This contamination was due to the fact that the cotton plug of this culture flask became moistened with the water of condensation which accumulated at this temperature. In the second experiment, a dish of calcium chloride was placed in the low-temperature incubators to absorb this moisture and to keep the cotton plugs dry.

From this experiment it was evident that *Phytomonas citri* passed through the various growth phase mentioned by Buchanan (5),

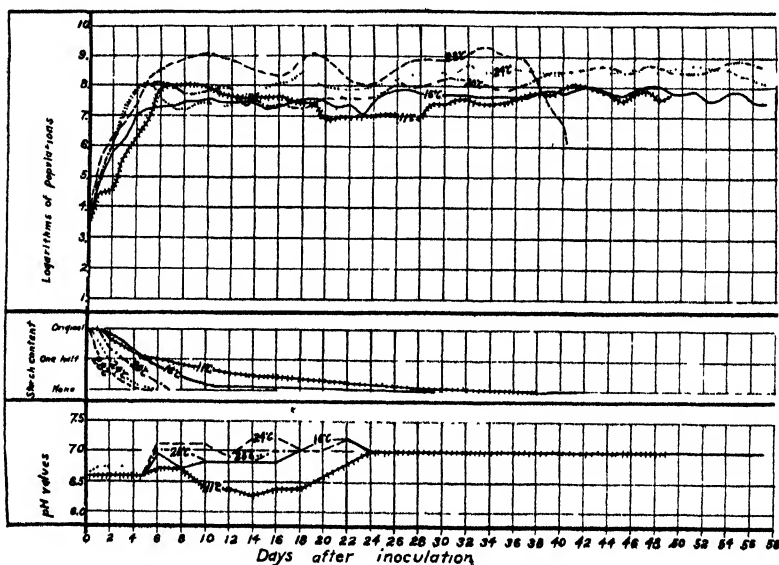


FIGURE 2.—Activities of *Phytomonas citri* in potato broth exposed at various temperatures. The upper set of graphs represents the rate of growth of bacteria at the temperatures indicated, the middle set the change in starch content, and the lower set the change in pH values in the same cultures. (Experiment 1.)

but an examination of these curves during the so-called logarithmic phase does not reveal much. Consequently, the test was repeated, as mentioned above, with observations at more frequent intervals.

The logarithmic values of the average of these populations as obtained from the duplicate flasks in the second experiment were recorded, and the results are graphically shown in the upper part of Figure 3.

An examination of these graphs shows that the logarithmic phase of the rate of growth at 29½° and 34½° C. occurred between the beginning and one and one-half days. The slope of these curves is very nearly the same. For the 21° and 25° temperatures the logarithmic phases occurred between the origin and the third day, with the slope of the 25° curve slightly greater than that of the 21°. If the portion of the 17½° curve between the first and the third days is considered

as the logarithmic phase, then its slope is about the same as that of the 21°. The curve for the 11½° culture shows a lag period of approximately four days. The slope of its curve between the fourth and the ninth days, or what may be called its logarithmic phase, is not so great as that of the others. If we accept Buchanan's (5) assertion that the logarithmic phase in the rate of growth curves is the most important, then the curves representing the rate of growth at 29½° and 34½° indicate that these temperatures

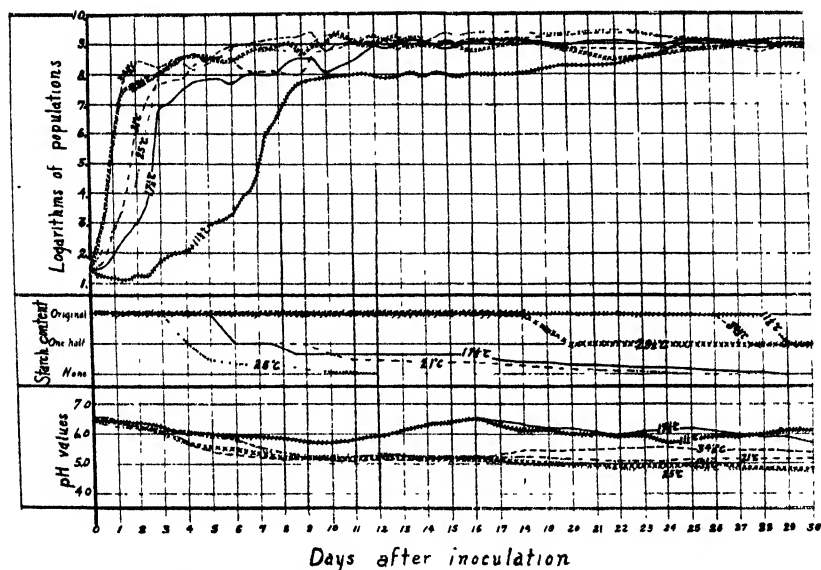


FIGURE 3.—Graphs representing logarithmic values of population averages and showing the activities of *Phytomonas citri* in potato broth exposed at various temperatures. The upper set of graphs represents the rate of growth of the bacteria at the temperatures indicated, the middle set the change in starch content, and the lower set the change in pH values in the same cultures. (Experiment 2.)

caused approximately the same rate of multiplication and were optimum for the growth of *Phytomonas citri*.

EFFECT OF TEMPERATURE ON DIGESTION OF STARCH

In order to determine the rate of digestion of the starch in the potato-broth cultures used in the above experiments tests were made of the samples at stated intervals with iodide solution which, according to Mellor (18a, v. 2, p. 99), was recommended by Stromeyer. When the broths were inoculated, 1 drop of the iodide indicator added to 6 c. c. of the broth gave a dark-blue color.

The reciprocals of the number of drops necessary to be added to 6 c. c. of the cultures to give the blue color were recorded and are shown graphically in Figures 2 and 3 for the first and second experiments, respectively. In the first experiment the higher the temperature the more rapid was the hydrolysis of the starch. In the second experiment the rate of hydrolysis of starch increased with the rise of temperatures up to 25° C., but decreased at temperatures of 29½° and 34½°.

EFFECT OF TEMPERATURE ON HYDROGEN-ION CONCENTRATION OF THE CULTURES

In the first experiment the hydrogen-ion concentration was determined by the colorimetric method. McAlvaine's buffer solutions as given by Clark (7) were used as checks for comparisons of colors from the indicators. The results were checked electrometrically. The pH values of cultures in the second experiment were determined by the quinhydrone method as developed by Biilmann and his associate (3). The results obtained on the days indicated in the figures are represented graphically in the bottom curves of Figures 2 and 3 for experiments 1 and 2, respectively.

The results obtained from these different tests fail to show any correlation between temperature and change in hydrogen-ion concentration.

LONGEVITY OF PHYTOMONAS CITRI IN STERILE AND UNSTERILE SOILS AND SOIL CONCOCTIONS

Because of the apparent discrepancies in the reported findings with regard to the longevity of *Phytomonas citri* in soil, the experiments described below were undertaken. The purpose of these experiments was to determine how long *P. citri* would live in sterile and unsterile soils and soil concoctions. Seven tests of this nature were made.

In the first test, run in series of 10, saw-grass muck from the vicinity of Hollywood, Fla., was used as sterile and unsterile soil and soil concoctions. The second test was similar to the first except that it was run in triplicate. The third was like the second except that soil preparations only were used. The fourth and fifth were like the third except that custard-apple muck from near Belle Glade, Fla., was substituted for saw-grass muck. The sixth was like the first except that sandy soil from Gainesville, Fla., was used. The seventh was run in duplicate with unsterile sandy soil of different moisture contents as indicated in Table 1.

TABLE 1.—Longevity of *Phytomonas citri* in sandy soil of different moisture contents

Series	Soil moisture	Viability of organism after number of days indicated *										
		1	2	3	4	5	6	7	8	13	20	27
1.	Per cent.											
	7.4	+	+	+	+	+	—	—	—	—	—	—
	10	+	+	+	+	—	—	—	—	—	—	—
	15	+	+	+	+	—	—	—	—	—	—	—
	20	+	+	+	+	—	—	—	—	—	—	—
2.	7.4	+	+	+	+	+	—	—	—	—	—	—
	10	+	+	+	+	+	—	—	—	—	—	—
	15	+	+	+	+	+	—	—	—	—	—	—
	20	+	+	+	+	+	+	—	—	—	—	—
	25	+	+	+	+	—	—	—	—	—	—	—

* + Signifies that the bacteria were still alive; — signifies that the bacteria were dead.

The soil concoctions were prepared by adding 2,500 gm. of the soil to 10 liters of sterile distilled water. The soil was prepared with 250 gm. to a sterile flask. In each series half of the preparations were sterilized and treated aseptically to guard against contamination before and after inoculation with *Phytomonas citri*.

The flasks were inoculated in each series in such a manner that approximately 27,000,000 bacteria were used per gram of dry soil. After the flasks were inoculated they were thoroughly shaken and placed side by side on a shelf on the shady side of the laboratory and incubated at room temperature, which ranged from 18° to 32° C.

Samples were removed from the test flask under aseptic conditions and tested for viability of *Phytomonas citri* by inoculating grapefruit trees. A record was kept of the trees which developed lesions, and the results are presented in Table 2. It is evident from these data that *Phytomonas citri* lived and perhaps multiplied in sterile sandy and muck soils, and in sterile sandy and muck soil concoctions for 150 days. In unsterile sandy soil and soil concoctions 3 days was as long as *Phytomonas citri* lived in any of the flasks except that containing 20 per cent soil moisture. In fact, in the majority of the sandy-soil flasks the organism did not live for 24 hours. Unsterile muck soil and soil concoctions did not kill the organism so readily. In some of these the bacteria were able to live for 13 days, although the majority were dead in 5 days or less.

A close examination of the records showed that there was a certain amount of variation in the length of time that *Phytomonas citri* lived in the various flasks in corresponding series of the different tests. This variation was not great, but it was thought desirable to determine whether or not it was due to the difference in the moisture content of the soils as they came from the field. Samples of sandy soil were so prepared by adding sterile distilled water to them that when 30 c. c. of a virulent potato-broth culture of *P. citri* was added a series was obtained with a moisture content as follows: 7.4, 10, 15, 20, and 25 per cent. The soil used was found to have a saturation point of 22 per cent. Samples from each flask were used to inoculate grapefruit leaves at stated intervals, and a record, as shown in Table 1, was kept of the trees that showed lesions.

From these results it is evident that there was no great difference in the length of time that *Phytomonas citri* lived in soils of different moisture content, and there appears, therefore, to be no correlation between soil moisture and longevity of the organism in the soil.

As a further test, grapefruit seeds were sown in pots filled with sandy soil and held at various constant temperatures. These temperatures covered a range from 11° to 38° C. Just before the seeds germinated the soil was inoculated with virulent cultures of *Phytomonas citri*. In no case, out of more than 600 seedlings so treated, did any sign of citrus canker appear. To check the possibility that the grapefruit seedlings might be immune to citrus canker as they germinated, one box of grapefruit seedlings was atomized with a broth culture of *P. citri* just as they were showing through the soil. Of more than 100 seedlings so inoculated, over 70 per cent showed citrus canker. Therefore it is evident that when the soil was inoculated with *P. citri* the organism died out very quickly in unsterilized soil.

TABLE 2.—*Longevity of Phytomonas citri in various Florida soils*

Ex- per- iment No.	Kind of soil	Treatment of soil	Viability of organisms after number of days indicated *														
			1	2	3	4	6	8	13	20	27	31	41	62	170	150	230
1	Saw-grass muck	Sterile concoction..... Unsterile concoction..... Sterile soil..... Unsterile soil..... Sterile concoction..... Unsterile concoction..... Sterile soil..... Unsterile soil..... Sterile soil..... Unsterile soil..... Sterile soil..... Unsterile soil..... Sterile concoction..... Unsterile concoction..... Sterile soil..... Unsterile soil..... Unsterile, 20 per cent soil moisture.	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2	do.		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
3	do.		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
4	Custard-apple muck		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
5	do.		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
6	Sandy soil		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
7	do.		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

* + signifies that the bacteria were still alive; — signifies that the bacteria were dead

SUMMARY

The rate of increase in population of *Phytomonas citri* grown in potato broth was greater at high than at low temperatures. For early growth $29\frac{1}{2}^{\circ}$ to $34\frac{1}{2}^{\circ}$ C. was optimum. However, at the end of 10 to 14 days the population at all temperatures for a given experiment was approximately the same. At 28° *P. citri* was dead after 40 days, although cultures at 11° , 16° , 20° , and 24° remained viable for 57 days.

The rate of starch transformation in these cultures increased with the rise of temperatures up to 28° C., but above this temperature the rate of transformation decreased.

There appeared to be no correlation between change in pH values of these cultures and the temperatures at which they were grown.

P. citri died out after 13 days in unsterilized Florida muck soils, and after 6 days in unsterilized sandy soils, at room temperature.

P. citri lived for at least 150 days in inoculated sterilized preparations of both muck and sandy soil at room temperature.

There appeared to be no correlation between the longevity of *P. citri* in sandy soil and the percentage of moisture in the soil.

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BACTERIAL STRIPE DISEASES OF SUGARCANE IN LOUISIANA¹

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INTRODUCTION

In the spring of 1927 leaf-stripe diseases, with symptoms similar to those of certain tropical bacterial diseases, occurred commonly on sugarcane throughout the entire sugar belt of Louisiana (2).² As no bacterial diseases of cane had been known to occur in Louisiana, investigations to determine the identity and importance of these troubles were started. As a result of these investigations, three distinct leaf-stripe diseases have been recognized in Louisiana. These are known as (1) the red stripe and top rot, (2) the mottled stripe, and (3) the white stripe. The first two are known to be caused by bacteria. The third one, the white stripe, is of considerable interest, as the stripe symptoms resemble very closely those of the leaf-scald disease of Java (8) and Australia (6). However, the disease seems to have but little economic importance in Louisiana, and as yet no organism has been isolated from the stripes. For these reasons it is not discussed in the present paper.

THE RED STRIPE AND TOP ROT DISEASE

Of the leaf-stripe diseases occurring in Louisiana, the one known as red stripe and top rot is the most important. This disease occurs on the leaves and leaf sheaths, producing red to maroon stripes (fig. 1, A), and in the stems producing a definite rot which ordinarily begins at the top of the plant.

On the leaves the first symptom of the disease is the appearance of water-soaked stripes which, especially on the margins, gradually become tinted with maroon. These stripes under favorable conditions develop rapidly, and by the end of four to five days reach a length of 15 to 40 cm. and a width of 1 to 4 mm. By that time they have taken on a distinct dark red or maroon color. Usually each stripe is bordered by a yellowish or chlorotic zone of about the same width as the stripe. The stripes are not confined to the leaves, but extend down on the sheath, where they usually become wider, sometimes reaching a width of 1 to 2 cm. Often in later stages many of the stripes coalesce, forming bands with alternate maroon stripes and chlorotic areas.

In the stem the first symptom of the disease is a slight reddening of the fibrovascular bundles in the growing point. Later the red color gradually extends down the stem, forming a red ring in cross section about one-quarter to one-half the distance from the rind to the center. The central portion of the stem rapidly takes on a water-soaked appearance, while the portion outside of the red ring changes very

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² Reference is made by number (italic) to "Literature cited," p. 267.

slowly. As the disease progresses this condition gradually extends to the extreme base of the stem. Finally deterioration of the water-soaked area occurs and the tissues break apart, leaving a hollow central cylinder. By this time, the leaves at the top of the plant are dead



FIGURE 1.—Bacterial stripe diseases on sugarcane leaves: A, Red stripe caused by *Phytomonas rubrilineans*. B, mottled stripe caused by *P. rubrisubalbicans*

and the upper part of the stem is badly rotted. The killing of the terminal bud also results in a sprouting and growth of the lateral buds. Like the main stem, these lateral shoots usually show a reddening of the central zone.

The red-stripe disease has been found widely distributed over the sugar belt of Louisiana on the following POJ³ varieties: 2727, 2714, 826, 2725, 213, 234, 36, 36-M, 979, and 228. The most serious natural infection observed occurred at Youngsville in August, 1928, one plantation showing a 10 per cent infection of top rot on POJ-2727 and 5 per cent on POJ-2714.

The organism causing red stripe was first isolated from leaves of POJ-213 cane collected at Youngsville on August 25, 1927. Since then many cultures have been obtained from both leaves and stems.

INOCULATION EXPERIMENTS

Inoculation experiments with pure cultures of the organism isolated from both leaves and stems have been consistently successful. Most of the inoculations have been made by introducing the inoculum into the growing point with a syringe. In practically all cases deep red to maroon stripes have appeared on the leaves and a large percentage of the stalks have developed top rot.

An inoculation test carried on in the summer of 1929 is an example of a series of such tests made with various varieties. In this test a susceptible variety, POJ-2725, was inoculated with five different cultures. Fifty plants were inoculated with each culture, and 50 plants were used for controls. After 12 days every plant of the 250 inoculated had red stripes, and 131 were developing top rot. On the controls no top rot or stripe symptoms developed around the needle punctures.

Inoculation experiments on different varieties have shown that POJ-2727, POJ-2725, and POJ-826 are very susceptible to the disease, while D-74 and the POJ varieties 36, 36-M, 234, and 213 are moderately susceptible. It is doubtful whether the disease would ever become serious in the latter varieties, but it could become quite serious in the former ones.

RELATION TO TROPICAL DISEASES

The red stripe and top rot disease as it occurs in Louisiana bears a close resemblance to diseases described from several tropical countries. Wakker and Went (7) described a top rot from Java and included a colored illustration which presents exactly the same symptoms as have been observed in Louisiana material. The evidence is very strong that the troubles are identical.

In Australia Cottrell-Dormer (1) and Wood (9) described a top rot and associated it with red stripes on the leaves. The disease was also produced by inoculating plants with mixed bacterial cultures. According to these authors, the Australian disease is confined to the upper joints of a stalk and does not spread through the stalk to the roots as is the case with the Louisiana disease. However, as the other symptoms are very similar, there is a strong likelihood that the diseases are identical.

In Hawaii Lee and his associates (4, 5) have described a red-stripe disease of Tip varieties and have named the bacterial pathogene *Phytomonas rubrilineans*. It is stated that the disease also attacks the stalks occasionally, but this point is not stressed. The stripes as described and illustrated are very similar to those produced by the

³ Proefstation Oost Java.

red-stripe disease in Louisiana. The cultural characters of the two organisms are also very similar. A few slight differences have been observed, but it is very doubtful whether these differences are important. As the varieties of cane used in the Hawaiian investigations are entirely different from those grown in Louisiana, it has not been possible to compare the susceptibility of different varieties to the two organisms.

While the evidence is not absolutely conclusive that the Louisiana disease is identical with any of the tropical diseases, for the present, at least, the name given to the Hawaiian pathogene, *Phytomonas rubrilineans*, will also be used for the Louisiana organism. Eventually investigators in the Tropics will be able to test out such susceptible varieties as POJ-2727 and will be able to determine definitely whether the tropical and the Louisiana leaf stripe and top rot troubles are identical.

THE MOTTLED-STRIPE DISEASE

The second bacterial disease of cane which has been observed in Louisiana is of less economic importance than the red stripe. To this disease the name mottled stripe has been applied.

The mottled-stripe disease as it occurs in Louisiana is primarily a disease of the leaf blade. The stripes (fig. 1, B) which are characteristic of the disease are predominantly red in color, though frequently white areas or white margins occur. This difference in color often gives the appearance of red on a white background and has suggested the specific name *rubrisubalbicans* for the causal organism. The stripes run parallel to the leaf veins and range in length from very short up to a meter or more, while the width is usually from 1 to 4 mm. One to many stripes may occur on the same leaf. When many stripes occur they sometimes coalesce, forming mottled red and white bands. A microscopic examination of the diseased tissue shows the presence of motile bacteria in great numbers, though bacterial exudation on the surface of the leaves has not been observed.

Of the canes commonly planted in Louisiana, the disease occurs most commonly on D-74. Natural infection on the varieties POJ-213, 36, and 234 is not common.

The organism causing the mottled-stripe disease was first isolated from leaves of D-74 collected at Baton Rouge, La., on June 28, 1927. Small sections of stripe tissue were sterilized in 1-1,000 mercuric chloride solution for 30 seconds, washed three times in sterile water, and then macerated in a small amount of Bacto-dextrose broth. Dilution cultures were then made with Bacto-dextrose agar and pure-culture isolations obtained. Since then the organism has been isolated many times.

LEAF-INOCULATION TESTS

Numerous inoculations have been made to test the pathogenicity of the organism, to trace the development of the leaf stripe, and to determine the susceptibility of different cane varieties. Usually the inoculations were made by covering the upper surface of the leaf with a suspension of the bacteria in dextrose broth and then scratching the inoculum into the leaf with a sharp sterile needle. Some inoculations were also made by introducing a quantity of the bacterial suspension into the terminal growing region with a syringe.

The first inoculations were made on July 8, 1927. With the culture isolated on June 28 inoculations were made on the basal portion of leaves of D-74 cane. Ten leaves were inoculated and 10 controls were scratched with sterile dextrose broth. At the end of 5 days the inoculated leaves showed stripes 3 to 8 inches long, and at the end of 27 days from 14 to 38 inches. None of the controls developed stripes.

Many similar tests with different cultures made during the summers of 1928 and 1929 gave practically the same results. The original culture isolated in 1927 remained virulent throughout this period.

While inoculations carried on in the summer were invariably successful, those made in the fall, when the plants had practically ceased growth and the weather was cooler, were not satisfactory.

VARIETAL SUSCEPTIBILITY

The susceptibility of different varieties of cane to the mottled-stripe disease was determined by inoculation tests. In the first test, started on August 2, 1927, nine of the most important varieties were used. One hundred leaves of each were inoculated and 50 held as controls. In this test the disease developed most rapidly on D-74, but Louisiana Striped, SC-12/4, and Louisiana Purple were also quite susceptible. On the POJ varieties 36, 213, 228, 234, and 979, practically no stripes more than 4 inches long developed, which indicated that these varieties were resistant. The controls remained free of stripes.

In another test started on August 14, 1928, 54 varieties and seedlings were used. Ten plants of each were inoculated by introducing the inoculum into the terminal growing region with a syringe. The varieties that had been used the previous year showed the same relative resistance as in the first test. Other varieties found to be susceptible were D-95, Crystallina, POJ varieties 2364, 2222, 2714, 100, and U. S. seedlings 434, 1419, 726, and 672. The varieties found to be resistant included L-511, Cayana 10, Badilla, Co-281, Uba, Kavangire, POJ varieties 1228, 2379, 139, 36-M, U. S. seedlings 559, 593, 666, 547, 689, 541, 676, 766, 665, 1532, 600, 590, 576, 1444, 1389, 510, and CP seedlings 130, 177, and 807.

STEM INOCULATIONS

In order to determine whether the mottled-stripe organism can attack parts of the plant other than the leaf, stem inoculations were made on 50 plants of D-74, and POJ-234 on August 4, 1927. A portion of an internode was covered with a 24-hour culture, and this inoculum was forced into the stem by puncturing with a sharp needle. For controls, portions of the stems of 40 plants of each variety were covered with sterile dextrose broth and punctured. On September 23, there was no noticeable difference between the inoculated plants and the controls. Aside from a slight reddening of a few fibrovascular bundles, no pathological symptoms were observed.

INOCULATIONS ON SEED CANE

A test started on August 9, 1929, was made to determine whether young cane plants can become infected readily from diseased seed cane. Thirty healthy single-eye seed pieces of the varieties D-74 and POJ-213 were sterilized for 10 minutes in a 1-1,000 mercuric chloride

solution. These were then washed twice in sterile tap water and immersed for 15 minutes in a 48-hour dextrose broth culture of the mottled-stripe organism. They were then planted separately in 6-inch pots which had been sterilized at 15 pounds pressure for 2½ hours. Thirty controls, not inoculated, were also planted. The pots were kept in the greenhouse and watered with sterile tap water.

Half of the plants were examined on September 7 and the remainder on November 11. On the latter date, the plants averaged 22 to 24 inches in height. The examination showed no differences between the plants from the inoculated and the control seed pieces. No mottled-stripe symptoms were observed on any of the plants.

As similar results were also obtained from field plantings made in the fall of 1927, it would seem that cane plants do not readily contract the disease from infected seed cane.

INOCULATIONS ON OTHER HOSTS

To determine whether the mottled-stripe organism is pathogenic to plants other than cane, 50 plants each of Johnson grass (*Holcus halepensis*), sorghum, corn, and cane were inoculated on August 4, 1928. After 18 days no mottled-stripe symptoms had developed on the corn plants. Of the sorghum inoculations all of the controls were negative, while 35 of the inoculated plants showed stripes ranging from one-half to 2 inches in length. The other 15 plants had been accidentally destroyed. Of the Johnson-grass inoculations the controls were negative, while 48 of the inoculated plants showed stripes one-fourth to one-half inch in length. The other two plants died from other causes. The sugarcane plants used as controls were negative, while the inoculated ones had stripes one-half to 4 inches in length. The test showed that the organism was parasitic to some extent on both Johnson grass and sorghum.

CULTURAL CHARACTERS

The mottled-stripe organism grows readily on most of the standard culture media.

On Bacto-dextrose agar plates (pH 7.0) at 30° C, the organism grows readily, the colonies reaching a diameter of 2 mm. in four days. By reflected light the colonies are circular, convex, entire, glistening, finely granular, viscid, milky-gray; by transmitted light, the margins are translucent and the centers are opaque and slightly buff tinted. In the medium surrounding the colonies, calcium oxalate crystals are abundant. Subsurface colonies after four days are small, lenticular, and opaque.

On Bacto-dextrose agar slants (pH 7.6) the growth after 24 hours is abundant, filiform, spreading at base, smooth, convex, glistening, gray-white, translucent to opaque, and slightly viscid. After seven days, growth is much more extensive and opaque. After the growth is loosened from the surface and agitated in distilled water, viscid strands remain suspended in the liquid for several minutes.

On Bacto-nutrient agar slants (pH 7.1) growth is at first scanty, filiform, glistening, smooth, translucent, gray-white, convex, and somewhat viscid. The growth is less abundant on this medium than on Bacto-dextrose agar.

On potato slants, after 24 hours, the growth is scanty, glistening, and light buff in color, and growth increases but slightly after further incubation.

On Bacto peptonized milk agar, after 24 hours, the growth is abundant, filiform, glistening, gray-white, raised, translucent, wet, and sticky. After 10 days the consistency is butyrous and the surface has a distinct iridescence, becoming more pronounced up to 24 days.

On Bacto-dextrose broth (pH 6.8), after 24 hours, a light lacy pellicle, which is easily precipitated, forms at the surface, and a heavy precipitate accumulates in the bottom of the tube. As the culture ages, new pellicles are formed and precipitated, increasing the amount of sediment. The latter becomes extremely viscid, and when agitated spirals upward in a ropy strand which slowly settles in a coil to the bottom of the tube when the agitation is stopped.

On Bacto-neutral red broth, clouding is moderate and the medium becomes eosin in color. The other characters are similar to those on Bacto-dextrose broth, though the sediment which develops is somewhat more viscid.

COMPARISON WITH RELATED BACTERIAL DISEASES

The mottled-stripe disease differs widely from the red-stripe disease as it occurs in Louisiana and as it has been described from Hawaii. The points of difference include the symptoms on the host, the susceptibility of various varieties, and the cultural characteristics of the causal organisms.

On the sugarcane plant, stripes of the mottled-stripe disease are red to mottled red and white, while those of the red-stripe disease are of a solid maroon color. The mottled-stripe disease does not attack the leaf sheaths or the stem, while the red stripe attacks both, causing a serious rot of the latter. Such varieties as POJ-36, 213, and 234 are very resistant to the mottled stripe but are susceptible to the red stripe.

The organisms causing the two diseases differ widely in certain important characteristics. The mottled-stripe organism produces capsules on dextrose media, does not liquefy gelatin, produces indol in 14 days, produces hydrogen sulphide in 3 days, exerts a rapid diastasic action on starch, and invariably produces an alkaline reaction in sucrose, dextrose, and lactose broth. On the other hand, the red-stripe organism, *Phytomonas rubrilineans*, does not produce capsules, liquefies gelatin, does not produce indol, produces hydrogen sulphide (does not according to Lee), exerts a slow diastasic action on starch (does not hydrolyze starch, according to Lee), and produces acid from dextrose (according to Lee).

The mottled-stripe organism has also been compared with the one which commonly causes a striping of Johnson-grass leaves in Louisiana. The latter organism is probably identical with the sorghum leaf-stripe organism, *Bacterium andropogoni* (3). The cultural characters of the two organisms are quite different, and the stripes on Johnson-grass leaves following inoculation with pure cultures are also different. The Johnson-grass organism invariably produces a broad stripe or blotch, as compared with the narrow stripe of the cane organism. (Fig. 2.)

TECHNICAL DESCRIPTION

As the organism causing the mottled-stripe disease of sugarcane in Louisiana seems to be distinct from other named bacteria, it is here described as a new species.

***Phytomonas rubrisubalbicans* sp. nov.**

A motile, short rod, slightly curved, with rounded ends and polar flagella; single, in pairs or rarely in short chains of from 2 to 6 cells; no spores; capsules present on dextrose media; aerobic but grows moderately under anaerobic conditions; gray-white to a light buff on agar; gelatin not liquefied; indol produced; hydrogen sulphide produced; starch hydrolyzed, no acid or gas produced from dextrose, lactose, maltose, saccharose, mannite, or malt extract; optimum temperature 30° C., optimum reaction pH 6.8 to 8; Gram-negative; not acid-fast;

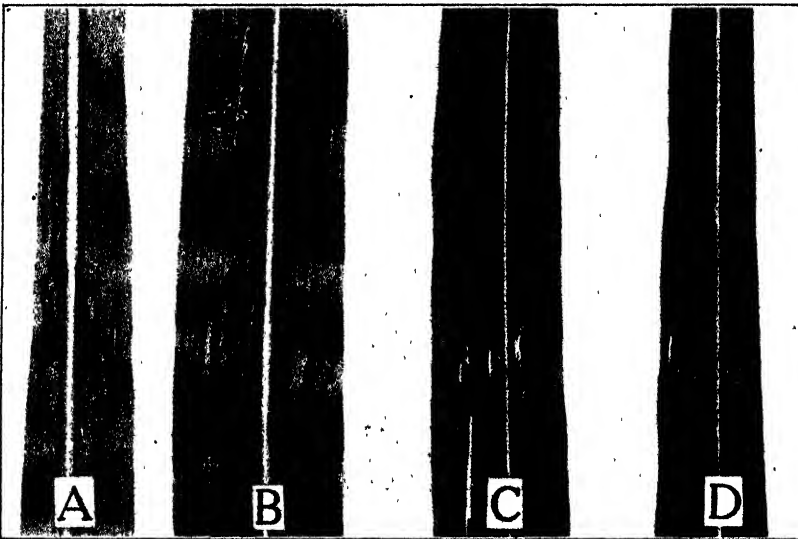


FIGURE 2.—Inoculation tests on leaves of Johnson grass: A, B, Leaves inoculated with *Bacterium andropogoni* isolated from Johnson grass; C, leaf inoculated with *Phytomonas rubrisubalbicans* from sugarcane; D, control, leaf scratched but not inoculated

pathogenic on leaves of sugarcane in Louisiana, causing the disease known as mottled stripe.

SUMMARY

Investigations carried on in Louisiana during a period of three years have shown that three leaf-stripe diseases of sugarcane occur there. Two of them are discussed in this paper. One of these, known as red stripe and top rot, is closely related to or identical with stripe and top-rot diseases in Java, Australia, and Hawaii. The disease is characterized by long, deep-red or maroon colored stripes on the leaves and by a rapid decay of the central portion of the stem. This decay is followed by the dying of the apical portion of the plant.

The evidence indicates that the organism causing red stripe and top rot is *Phytomonas rubrilineans*. It was isolated many times from infected leaves and stems. Inoculation experiments on leaves and stems were invariably successful. Inoculation experiments with pure cultures of the red stripe and top rot organism have shown that the POJ varieties 2727, 2725, and 826 are very susceptible,

while the D-74 and POJ varieties 36, 36-M, 234, and 213 are moderately susceptible.

The other disease, named mottled stripe, is characterized by the presence of long stripes on the leaf blades. These are predominantly red in color, though a mottled red and white condition often occurs. An organism, described as *Phytomonas rubrisubulbicans*, sp. nov., was isolated many times from tissues showing mottled-stripe symptoms. Inoculation experiments on the leaves of cane with pure cultures were invariably successful. Inoculations in stems of cane were negative. As a result of the inoculation experiments, the following varieties of cane have been shown to be very susceptible to the mottled-stripe disease: D-74, POJ-2364, POJ-2222, POJ-2714, POJ-2727, Crystallina, D-95, SC-12/4, Louisiana Striped, Louisiana Purple, and POJ-100. Among the varieties showing considerable resistance to the disease, though not immune, were POJ varieties 36, 36-M, 234, 213, 228, 979, Co-281, and CP varieties 130, 177, and 807.

The mottled-stripe disease was transmitted to Johnson grass (*Holcus halepensis*) and sorghum, but not to corn.

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CORTICIUM CENTRIFUGUM, A HETEROTHALLIC PATHOGENE OF APPLES¹

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INTRODUCTION

In 1903 Eustace (13)³ reported a previously undescribed rot of apples. This disease, formerly called *Hypochnus* rot (9), is termed "fisheye" rot in this paper. The rot was found in New York only, and on but two varieties of apples, Baldwin and Rhode Island. In each instance the decay followed scab and appeared as brown, sunken, circular lesions which varied in size. Isolations from the necrotic tissue yielded a fungus that was designated as *Hypochnus* sp. The pathogenicity of the fungus was demonstrated by inoculating apples of the same variety as those from which it was isolated, as well as 33 additional varieties and 5 varieties of pears. It was also ascertained that when infected fruit was held in moist chambers it gave rise to superficial delicate dirty-white mycelial mats.

As previously reported (9), the increasing prevalence of the so-called *Hypochnus* rot of apples has attracted attention on many eastern markets. Diagnoses based on the isolation and identification of the parasite have shown that the disease is more widespread than was formerly reported. On the markets the decay has been found on Baldwin, Rhode Island, Roxbury, and Ben Davis apples from New York; Winesap, Ben Davis, and Willow apples from southern Illinois; Winesap, Jonathan, and Rome apples from both Wenatchee and Yakima, Wash.; Jonathan and Winesap apples from Idaho; Yellow Newtown apples from Oregon; and Ben Davis apples from Virginia.

COMPARISON OF SYMPTOMS OF FISHEYE AND OTHER ROTS OF APPLES

On apples grown in New York the so-called *Hypochnus* or fisheye rot usually follows scab (pl. 1, A, C), although it has been found many times when no scab was discernible on the fruit (pl. 1, B). When following scab, it appears externally as a tan to brown circular area. (Pl. 1, A.) Individual lesions are usually less than 1 inch in diameter (pl. 1, A), but sometimes they coalesce to form larger areas (pl. 1, C). The injured area is generally markedly depressed. The

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³ Reference is made by number (italic) to Literature Cited, p. 292.

depression and the color change clearly delimit the necrotic region from the normal tissues. The enlargement of the lesion is uniformly centrifugal, resulting in the maintenance of the point of inception, commonly a small scab lesion, in the center of the decayed area.

The lesions are cone shaped, and the depth is usually approximately equal to the diameter on the surface of the fruit. The sub-epidermal tissues are browned and disintegrated. The tissues are characteristically dry and somewhat stringy, giving to the lesion as a whole a dry, spongy consistency. When infected apples are held under sufficiently humid conditions, as in moist chambers, the mycelium spreads over the surface of the apples, radiates uniformly from the lesions, and forms a characteristic, delicate, appressed, white mycelial mat. (Pl. 1, E.)

Eustace (13, p. 125) pointed out the similarities in macroscopic appearance of this rot and the pink-mold rot caused by *Cephalothecium roseum*, and presented the differences as follows:

On fruit affected with *C. roseum* there is usually a conspicuous white or pinkish growth of the fungus in the center of an affected spot; whereas this new fungus does not show at all conspicuously on the surface of a decayed spot until made to do so by artificial conditions. On fruit, *C. roseum* is a very shallow growing fungus, penetrating the tissue not much more than an eighth of an inch, while this new fungus grows much deeper and in its late stage extends to the core.

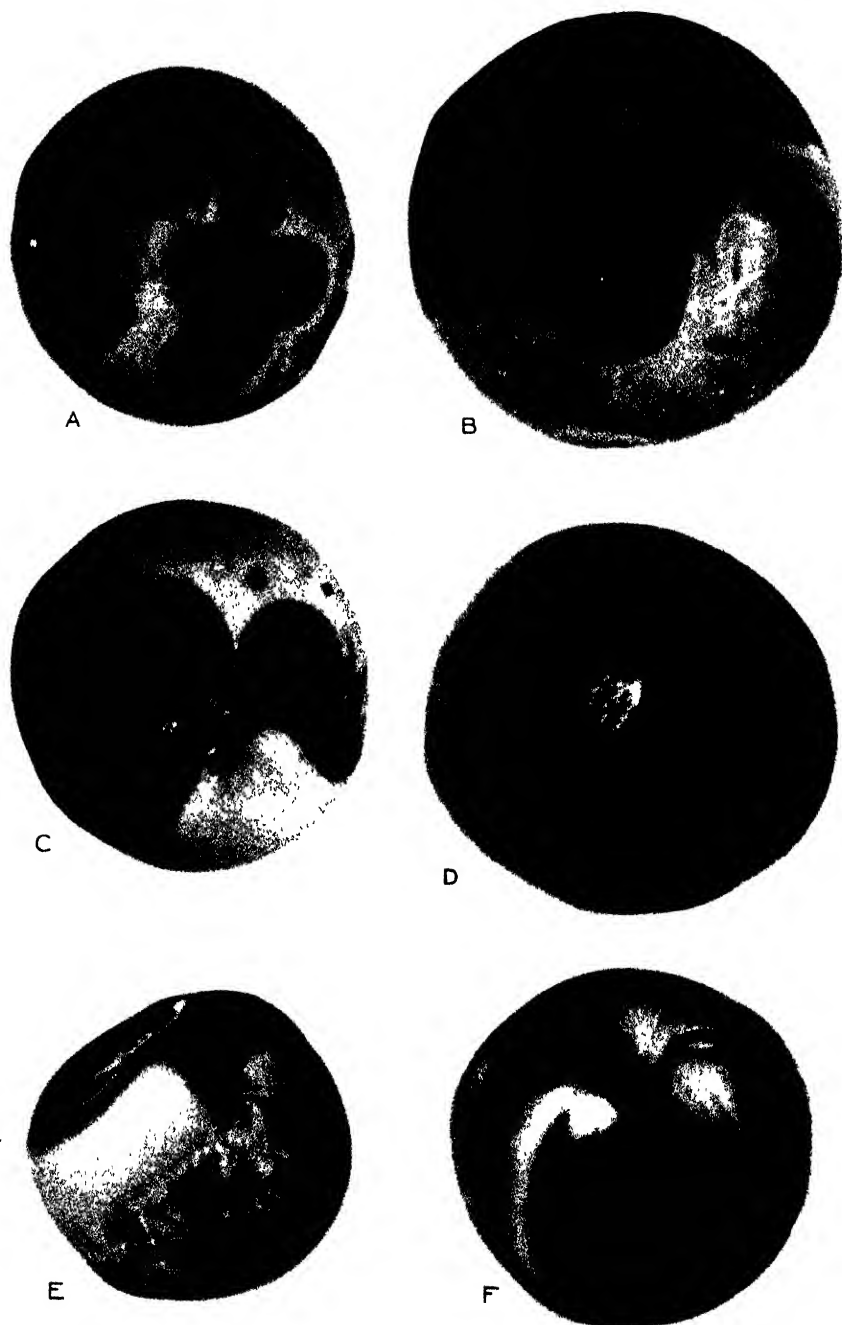
The mycelium of the fisheye-rot fungus has been seen growing superficially on barreled apples from the State of New York, in which there was a high degree of humidity. When apples upon which the mycelium was growing were removed from the humid atmosphere necessary for this external growth of the fungus, the mycelium quickly disappeared.

A somewhat analogous condition occurred in the Northwest in that the symptoms of the new rot agreed very closely with those of other rots of northwestern apples that have received much attention. To make this clear, a brief discussion of the field rots of northwestern apples is necessary.

The first apple rot having its inception in the orchards in the Northwest was reported in 1900 by Cordley (10), and was found to be very prevalent on northwestern apples inspected on eastern markets. The disease was named anthracnose or northwestern anthracnose (*Neofabraea malicorticis*). The outstanding characteristic of the apple rot is the tan center and dark border of the skin over the lesion. The disease is for the most part confined to the more humid sections of the Northwest.

About 1920 a rot of similar appearance was found on the markets on northwestern apples shipped from localities said to be free from anthracnose. Isolations from these specimens yielded a fungus that did not sporulate. Because of the similarity in appearance of the lesions to those caused by anthracnose and the obvious difficulties involved in classifying the sterile pathogene, the rot was called false anthracnose.

In 1925 Zeller and Childs (47) reported a new apple disease in the Northwest. The fruit-rot phase of this disease, which also has its inception in the orchards, was named bull's-eye rot. The symptoms on the fruit were found to be very similar to those of anthracnose and false anthracnose, and the rot was thought to be the same as the false



Apples affected with fisheye rot caused by *Corticium centrifugum*

A-C, Baldwin: A, Small lesions following scab and responsible for name fisheye rot; B, large depressed lesion not following scab; C, large irregular lesion formed by coalescing of small lesions. D-F, Winesap: D, Small lesions, showing degree of depression; E, advanced stage, showing appearance of superficial mycelium after exposure to dry air; F, multiple infections, showing coalescence.

anthracnose reported on the markets. However, it is noteworthy that the false-anthracnose organism has been found to be consistently sterile, whereas the bull's-eye rot pathogene (*Gloeosporium perennans*) fruited readily.

In the process of making isolations for the purpose of diagnosing decays of northwestern apples found on eastern markets, each of the three organisms causing the three rots (anthracnose, false anthracnose, and bull's-eye rot) has been found many times. In addition to these three forms there was found, as reported by the writer (9), a fourth pathogene that had clamp connections and was shown by subsequent cultural studies to be morphologically identical in both its vegetative and fruiting stages with the nodose septate fungus previously isolated from eastern apples. The lesions from which these isolations were made were quite similar to those of the three rots already described on northwestern apples. As the causal organism of the new rot seemed identical with the pathogene of the apples from New York, it was felt that the same name should be used. As heretofore stated, the rot is most often found in apples from New York, following scab. The dark circular scab spot in the center, bordered by the tan depressed ring of discolored apple skin, presents a somewhat cyclike appearance. (Pl. 1, A.) Members of the trade on eastern markets had used the name fisheye rot to designate this type of lesion. Since the name already had some standing and is descriptive, it has been adopted and is being used as a common name for the disease.

It is seen, in summarizing, that four apple rots having the same general appearance have been reported on northwestern apples. The lesions consist of cone-shaped necrotic tissues. The point of the cone extends into the flesh of the fruit, and the base is formed by the discolored epidermis covering the lesion. The discolored epidermis is usually circular in shape, having a tan center and a dark border. (Pl. 1, D, F.) The color contrast between the central area and the border is often more pronounced on red, well-blushed surfaces than on yellow or lighter areas. The similarity between the four decays is so pronounced that in many instances isolations and microscopic studies of the fungi are necessary for diagnosis. However, some macroscopic differences have been observed. Bull's-eye rot and anthracnose are very similar. The necrotic tissues are moist and mealy; small lesions are not usually depressed; and superficial growth of the mycelium is very scanty and tufted, if present at all. Both fisheye rot and false anthracnose afford contrasts to these characteristics. In the latter two diseases the necrotic tissues are usually dry and spongy, the lesions even when very small are commonly depressed (pl. 1, D, F), and if the mycelium appears on the surface it is appressed and matted (pl. 1, E).

PREVALENCE OF FISHEYE AND OTHER ROTS OF APPLES

Any estimate of the relative prevalence of the four field rots found on apples grown in the Northwest is obviously confused by the difficulties of diagnosis. While it is felt that bull's-eye rot and anthracnose are much more prevalent in transit and storage, it is obvious that the greater publicity given these two diseases has somewhat obscured the presence and prevalence of both false anthracnose and fisheye rot.

Winesap apples from the Northwest showing the characteristic symptoms (pl. 1, D, E, F) were collected from a large number of fruit stores in late spring and cultured in the laboratory. These specimens showed almost 100 per cent false anthracnose and fisheye rot. In addition to this rather restricted investigation, many confirmatory isolations have been made in late March and April from apples mailed to Chicago by inspectors of the Bureau of Agricultural Economics, United States Department of Agriculture. This cultural work, together with observations made on some 10 or 12 other eastern markets during the last three years, indicates that both false anthracnose and fisheye rot are consistently present in northwestern apples in late spring. The percentage of decay in any given lot of fruit at this time of year is usually but not always small. The following quotation accompanied six Rome apples mailed to the writer for diagnosis:

Car M. D. T. [Merchants Dispatch Transit Co.] 95826 out of Benton City, Wash., on October 17. In storage in Minnesota till March 21, arrived Detroit 25th or 26th of March. Shows decay ranging from 4 to 60 per cent, averaging 29 per cent, according to M. C. [Michigan Central] inspection records. Inspection restricted to top tier boxes and doorway.

Cultures made of these apples showed that both false anthracnose and fisheye rot were present. In fact, both diseases were found on one of the apples. The inspector of the car stated that the specimens sent to the laboratory were representative of all the decay found.

Inasmuch as pink-mold rot and the so-called *Hypochnus* rot, both found on apples grown in New York, are so similar in their macroscopic symptoms, and as pink-mold rot has received a great deal more attention, the importance of so-called *Hypochnus* rot has been overlooked. In the last three years approximately 100 cultures have been made from lesions on apples grown in New York that fitted the description of the rot as given by Eustace (13). In most of these cultures the fungus isolated fitted accurately, in both the vegetative and fruiting stages, his published description of the causal organism. In other cultures made from the same type of lesion, sometimes from the same apples, a macroscopically similar growth occurred. Microscopic examination, however, disclosed differences. Further treatment of this phase of the work is confined to later discussion in this paper. The morphological and pathogenic similarities are rather conclusive evidence that the pathogene isolated repeatedly from apples from New York inspected on many eastern markets and the fungus described by Eustace (13) are the same organism.

An examination of many cars of barreled apples from New York has shown as high as 25 per cent of the lot to be infected with fisheye rot. Diagnoses in these cases were confirmed by culturing representative specimens. It has been found that fisheye rot is much more common late in the storage season than at any earlier time.

METHOD OF INFECTION

Although very few data are at hand, the studies and observations that have been made indicate that the inception of the fisheye decay occurs in the orchards. The fungi comprising the group to which the form that causes fisheye rot belongs are, in the main, saprophytes that are found on stumps and dead branches. In the labora-

tory the causal fungus has grown well on apple twigs and stems. The writer found it growing over the surface of apples in an orchard near Wenatchee, Wash. The superficial growth was characteristic, and microscopic examination of the hyphae showed the clamp connections. It is considered quite probable that the organism establishes itself in tissues of the mature fruit that are either dead or at a low ebb of life, for example, stems, calyxes, or lenticels, in the orchard, and develops after the fruit is shipped. It has been found many times on the surface of eastern and western apples that were not decayed.

A study was made to test the assumption that the causal fungus was present in the stems of apples. Fifteen sound Winesap apples were selected from a car, inspected on the Chicago market, in which fisheye rot was found. The apples were not sterilized but were held in moist chambers in the laboratory for three weeks. In nine of the specimens the nodose septate fungus grew out from the stems and over the shoulders of the apples. In this and similar observations no decay resulted from the superficial mat. This is taken as evidence that an opening is necessary for infection. Eustace (13) obtained negative results when he placed inoculum on the surface of apples, and concluded that the fungus could not penetrate the sound epidermis. Temperature studies have shown that the decay does develop under commercial cold-storage conditions. It seems highly probable that the lesions found on fruit in the markets have resulted from an incipient infection that was present, but invisible, when the apples were packed. In brief, it is felt that the disease has its inception in the field and that it develops but does not spread in transit or storage.

CULTURAL STUDIES

From the isolations previously discussed, some 50 strains of the pathogene, considered representative of the different localities mentioned, have been carried in the laboratory. The studies of the cultures were made in a comparative way. Thus, although initial studies were made on strain No. 211-C, isolated from Baldwin apples grown in New York, the morphological and physiological characteristics that were determined for this form have been found to be duplicated by other strains from New York and from all other localities reported. Some few strains have shown differences in culture, but the variations are small and mostly of a physiological nature. Five cultures from New York apples differed slightly from the other New York forms. No differences between strains have been found to be correlated with the localities from which they came. Sufficient studies to determine whether all the forms isolated should be referred to the same species have not been completed. However, as will be more fully discussed later, it is felt that enough evidence has been obtained to justify the conclusion that the same species is present in all the different localities from which the diseased specimens were shipped. The present paper deals only with the similar strains found in the various localities; a discussion of the variants is postponed.

The hyphae were smooth and white and had clamp connections, commonly one at each septum. (Fig. 1, B, C, E.) The hymenium consisted of loosely interwoven hyphae identical in appearance with those shown in the drawing of Eustace (13, *pl.* 2). The clavate

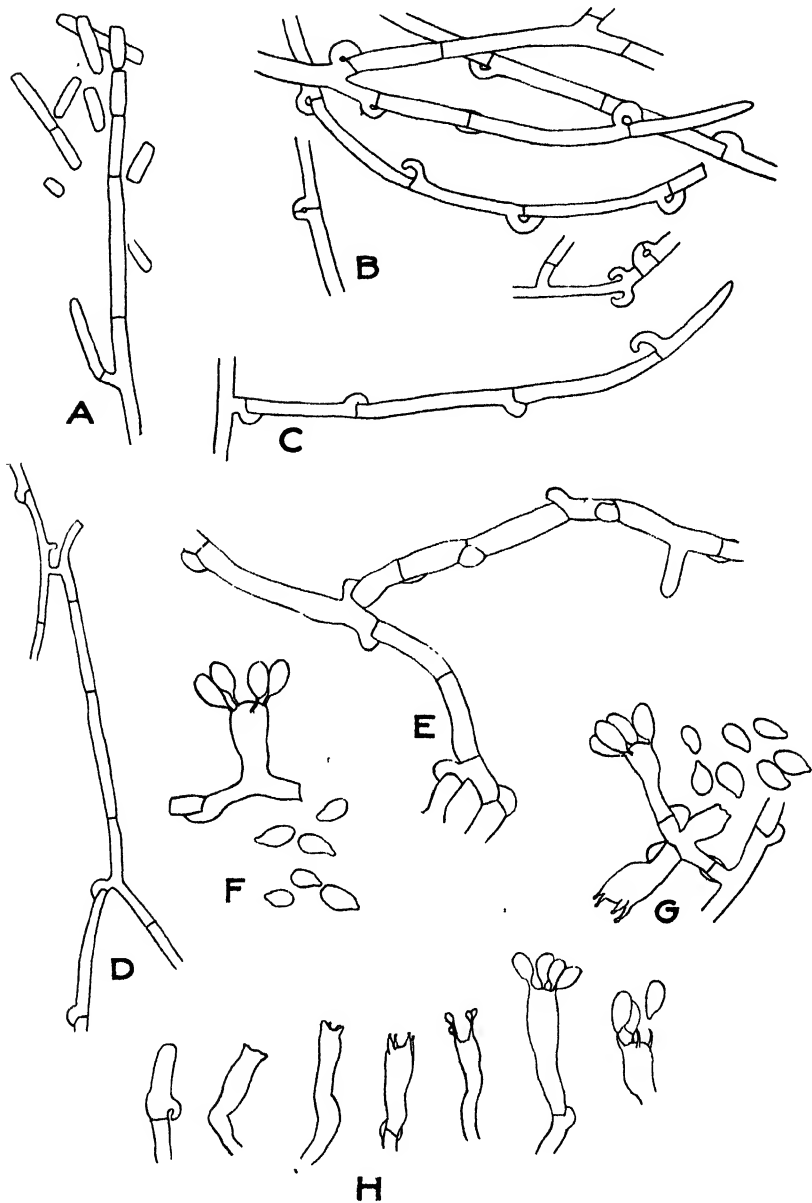


FIGURE 1.—Mycelia and spores from different strains of *Corticium centrijugum*. $\times 700$: A, Haploid mycelium and oidia of single-spore strain No. 14; B, hyphae showing irregular and incomplete clamps in the mycelium over the inoculum of single-spore strain No. 14, six days after crossing with No. 266-A (fig. 2, A); C, hypha showing normal and incomplete clamps 2 cm. behind the inoculum of strain No. 14 in the new mycelium that had developed six days after crossing with No. 266-A (fig. 2, A); D, anastomosis of hyphae present at place of contact between strains Nos. 274 and 2 (fig. 2, B); E, hyphae from perfect strain No. 211-C, isolated from apples grown in New York; F, basidium and spores from No. 211-C; G, portion of hymenium and spores from perfect strain produced by crossing two sterile western strains (Nos. 266-A and 274); H, stages in the development of basidia, sterigmata, and spores.

basidia, arising directly from the hyphae and bearing four spores on sterigmata (fig. 1, F, G, H), agree in manner of origin and development as well as in form and size with the previously published characterization. Further agreement is found in the length and number of the sterigmata and the number, shape, and size of the spores borne at the distal extremities of the basidia. (Fig. 1, F, G, H.)

The pathogene grows very well on all the common culture media. It also grows well on apple twigs, apple stems, and bean pods. Spore formation has been more copious on bean pods and string-bean agar than on any other substrate tried. While some success has attended efforts to induce fruiting, enough failures have occurred to prevent the assumption that sporulation may be produced at will. The pronounced sensitivity of the vegetative phase to changes in humidity and moisture content of the medium, and other observations as well, indicate that the degree and rate of desiccation to which the fungus is subjected are important factors in sporulation.

Although temperature studies have not been completed, it has been found that on Baldwin apples from New York and Winesap apples from Washington an appreciable growth occurs at cold-storage temperature (32° F.). Apples were inoculated subepidermally with small portions of mycelium and placed in a commercial cold-storage room the same day. The final observations made six weeks later showed that the pathogene had established itself and produced lesions averaging 15 to 20 mm. in diameter. The optimum temperature for growth on media lies between 65° and 75°. The maximum temperature for growth has not been determined, though slow growth occurred in Petri dishes on corn-meal agar at 90°. Inasmuch as humidity controls and accurate temperatures were not available, except in the commercial cold-storage room, the temperature studies were postponed.

Single-spore cultures of the fungus were made as follows: Portions of the hymenium of a fruiting culture that had been made from a Baldwin apple from New York and had been carried in the laboratory for two years were transferred under aseptic conditions to tubes of sterile water, shaken to remove spores, and further dilutions made. The spore dilutions were poured over thin layers of clear agar in Petri dishes. After the water had been absorbed so that the spores were lodged on the agar, the Petri dishes were inverted on the stage of a microscope for examination. When an isolated spore was found its location was marked with a circle of india ink drawn on the bottom of the Petri dish. Next, the agar within this area and the adhering spore were transferred to another dish containing a thin layer of clear agar. The transferred spores were then examined macroscopically at intervals until after germination to make sure that each colony was from a single spore. When this had been ascertained, transfers were made to test tubes of agar and the strains were numbered. In this manner 70 single-spore cultures were made. While the mycelium of most of the single-spore cultures was microscopically similar to the parent culture, microscopic examination disclosed differences. The hyphae were smooth and white but had ordinary septa instead of clamp connections. None of the cultures formed either basidia or spores, but oidia were formed copiously by segmentation of hyphae. (Fig. 1, A.) Oidia have not been found in any of the strains that had clamp connections and formed basidia and spores, but have been found in all single-spore cultures.

Cultural studies reveal a variation in vigor among the different single-spore strains. Some are almost as vigorous as the parent strain, though the perfect or parent strain is on the whole more vigorous than any of its sterile progeny. One measure of the relative vigor in the different single-spore strains is shown by the rate of growth. Table 1 shows the results of a comparative study. Bean agar contained in Petri dishes was inoculated with the parent culture, each of its single-spore strains, and the two western sterile strains (Nos. 266-A and 274), respectively, and kept at room temperature. The cultures were allowed to grow two days to establish themselves before initial measurements were taken. The total radial increase and average daily increase during the next five days were recorded.

TABLE 1.—Comparative growth rates of a perfect strain (No. 211-C) and of sterile strains of the fisheye-rot fungus

Culture No.	Genotype	Growth in 5 days (mm)		Culture No.	Genotype	Growth in 5 days (mm)	
		Total	Average			Total	Average
211-C	AaBb	32.5	6.4	9.	Ab	12	2.4
1.	AB	24	4.8	10.	do.	5	1.0
6.	do.	5	1.0	14.	do.	14	2.8
15.	do.	8	1.6	16.	do.	13	2.6
18.	do.	5	1.0	21.	do.	14	2.8
23.	do.	13	2.6	27.	do.	5	1.0
4.	ab	7	1.4	28.	do.	10	2.0
12.	do.	18	3.6	2.	aB	4	.8
13.	do.	10	2.0	3.	do.	3	.6
17.	do.	7	1.4	7.	do.	10	2.0
20.	do.	12	2.4	8.	do.	18	3.6
22.	do.	5	1.0	11.	do.	10	2.8
25.	do.	(*)	(*)	19.	do.	5	1.0
26.	do.	4	.8	24.	do.	12	2.0
29.	do.	3	.6	266-A.	aB	4	.4
30.	do.	3	.6	274.	Ab	5	1.0
5.	Ab	13	2.6				

* Died.

The parent culture, No. 211-C, grew more rapidly than any of its progeny or than the two western sterile strains. Duplicate plates showed that the variation in rate of growth is consistent for the individual strains. It will be noted, however, that the rate of growth does not vary with the genotype. When two strongly mating haplonts are paired the spread of the resultant colony proceeds at a faster rate after the diploid condition is initiated than it did while its two components were still in the haploid state. An example is seen in a comparative study of the amount of new growth in two colonies shown in Figure 2, A, 2 and 14. The faster rate of growth seems correlated with the perfect or diploid condition.

While the longevity of the fungus in culture has not been studied, it has been found that the diploid mycelium can live as long as six months without transference, though many cultures of the single-spore strains die in a few weeks if not transferred to fresh culture medium. Observations indicate that there is a correlation between mortality and the sugar content and moisture of the substrate.

The absence of clamp connections in the hyphae of the single-spore cultures indicated that the fungus was heterothallic. Pairing of the monosporous strains was therefore begun.

RESULTS OF CROSSINGS

CROSSING OF SINGLE-SPORE CULTURES OF FISHEYE-ROT ORGANISM

Thirty single-spore cultures of the fisheye-rot organism were selected arbitrarily for crossing studies. The cultures were numbered 1 to 30 consecutively. Culture No. 1 was paired with each of the 29 other cultures. The pairings were made on bean agar in test tubes by placing small pieces of inoculum of the two strains close together in each tube. Checks were made by transferring each of the 30 strains individually to other tubes. Microscopic examination of the cultures two weeks later showed that the crossing of six of the strains—Nos. 4, 12, 13, 17, 22, and 25—had resulted in clamp formation, but that no clamps were found in any of the other tubes nor in any of the checks. Basidia and spores were found in each of the cultures that had formed clamps, but they were not found in any of the other cultures. This is in keeping with the findings of others.

Since Bensaude (1) demonstrated heterothallism in the Hymenomycetes in 1918 by her work on *Coprinus finetarius*, a comprehensive study of the condition has been made by many workers. In such studies definite criteria by which the haplonts and diplonts may be distinguished are essential. Clamp connections have become the accepted criterion in distinguishing between the haploid and the diploid condition. The monosporous mycelia of heterothallic Hymenomycetes has been found to be uninucleate and devoid of clamps, where-

as the pairing of compatible monosporous strains results in a mycelium with paired nuclei that has clamps (1, 20). Heterothallism is therefore evidenced by the presence of clamps in the compound mycelium and the absence of clamps in the monosporous mycelium. Cytological studies of heterothallic forms (1, 25) have demonstrated that two monosporous uninucleate mycelia, by anastomosing, give rise to the compound mycelium with paired nuclei. In many Hymeno-

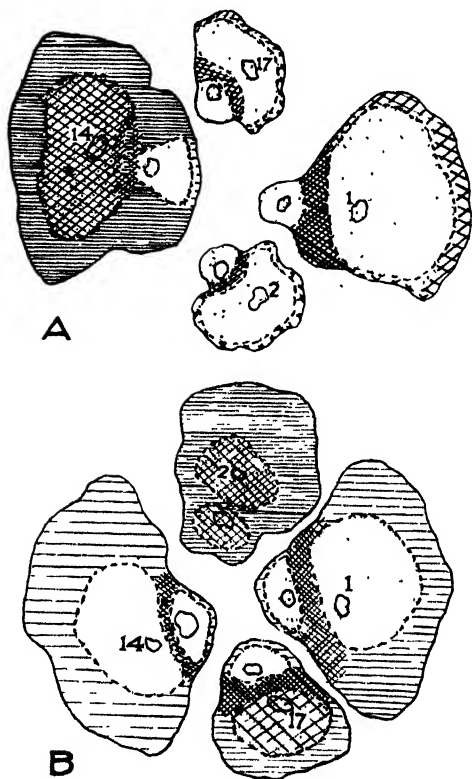


FIGURE 2.—Diagrams showing areas of clamp formation in crosses between two sterile western strains (Nos. 266-A and 274) and four sterile eastern strains (Nos. 1, 2, 14, and 17). The numeral on each colony is the number of the eastern strain with which the western strain was crossed. (Table 5.) Shading with horizontal lines denotes normal number of clamps; crosshatching denotes normal and irregular clamps; dotted areas denote oidia present; broken lines mark separation of old growth from that developed after crossing. In each cross the inoculum of the western strain is nearest the center of the diagram. A, Crosses of No. 266-A with the eastern strains. B, Crosses of No. 274 with the eastern strains.

mycetes the haplonts form oidia but the diplonts do not. However, some variation has been found in this, so it is not always a reliable criterion. Although fruiting is usually confined to the binucleate phase, Kniep (19) has found that haplonts of *Schizophyllum commune* produced fruiting bodies and spores morphologically similar to the fructifications of diplonts, though all the spores produced on the sporocarp were of one sex. Similar findings have been reported by Kniep (20), Vandendries (43), Brunswik (2), Zattler (46), and Hanna (17). Therefore in the work presented here, although the formation of oidia and basidiospores has been taken into consideration, the basic criterion has been the presence or absence of clamps.

Inasmuch as culture No. 1 grew more vigorously than any of those with which it had mated, it was designated as a plus strain and those that had crossed with it were recorded as minus strains. Culture No. 1 and culture No. 17, the latter one of the minus strains, were then paired with each of the 28 other single-spore cultures in the manner previously described. Checks of each culture were made as before. Examination three weeks later proved that culture No. 1 had again mated with each of the six minus strains. Clamps were also found in the crosses between culture No. 1 and cultures Nos. 20, 29, and 30, which were therefore recorded as three additional minus strains. Since clamps were present in the crosses between culture No. 17 and cultures Nos. 1, 6, 18, and 23, these two sets of pairings thus resulted in the segregation of four plus strains and nine minus strains. No clamps were found in any of the other tubes nor in the checks.

It had been necessary to duplicate some of the pairings because the cultures made as checks did not grow. The failure of these strains to grow in the paired cultures would doubtless be overlooked because the two pieces of inoculum were placed in contact with each other and the mycelium from the more vigorous of two included strains could easily cover the inoculum of the other strain.

Consequently, a new technic was employed, and pairing between cultures Nos. 1 and 17 and each of the other single-spore strains was repeated in the following manner: Inoculations were made on sterilized string-bean pods, two pods, one much shorter than the other, being placed in each test tube. A few cubic centimeters of distilled water was added to each tube to prevent too rapid desiccation. In all inoculations with cultures Nos. 1 and 17 these strains were placed on the upper end of the short bean pod and the strain with which they were paired was placed on the upper end of the long pod. The cavities at the tops of the broken pods afforded a convenient receptacle for the inoculum. Care was exercised in the inoculation to insure the presence of but one of the strains on each pod. The distance separating the two pieces of inoculum thus afforded ample opportunity for checking the viability and growth of the less vigorous strains before the mycelium from the vigorous strains had developed far enough to cover them.

Examinations for clamps were made a week later, when in all tubes the mycelium of both strains had reached the lower ends of the bean pods and formed a profuse mat at the point of contact. Mounts for study were made from the bean tissue at the region of contact. Clamps were found in all crosses in which they had occurred previously and in two additional ones. Culture No. 15 crossed with No. 17, and

It is seen that the progenies of the nodose septate form are differentiated into four types as demonstrated by their ability to fuse and form clamps. Kniep (23) listed 15 species of Hymenomycetes, among them *Corticium serum*, for which this phenomenon has been reported. This occurrence of four types of spores has been variously explained. In general it is interpreted as a sex phenomenon. Prell (34), however, favored the view that this and similar phenomena in fungi which he designates as "Aëthogametic" do not involve sex differentiation. Buller (6), Newton (29, 30), and Mounce (27, 28) interpreted it in terms of sexuality and designated such a fungus as "quadriseexual." They used the genetic notation introduced for such situations in fungi by Kniep (19, 20, 21).

Kniep (21) postulated that copulation of haplonts in Hymenomycetes is determined by genes which may occur as multiple allelomorphs. Thus, in such results as those recorded in Table 2, one is dealing with a haplodioecious fungus with two pairs of such allelomorphs localized in four different chromosomes. This type of fungus is designated as of the "tetrapolar" or "dihybrid" type. At first Kniep designated these genes as "sex factors" (19, 20, 21). Later, in harmony with the suggestion of Prell (34), Brunswik (2), Oehlkers (31), and others, he (22) dropped this term and, following the suggestion of Oehlkers, designated them as copulation factors. Consequently he replaced the phrase "pluripolar sexuality" by "pluripolarity of copulation-determining factors." He made the fundamental assumption that the presence of identical copulation factors prevents copulation. Thus *AB* can copulate only with *ab*.

Brunswik (2) has interpreted identical phenomena in his experimental material by assuming the operation of sterility factors. He assumed that autogamy is the fundamental process in both monoecious (homothallic) and dioecious (heterothallic) fungi; that is, both have the same genotypic constitution so far as sex is concerned. Heterothallism is determined by the addition of inhibiting or sterility factors, and mutability and allelomorphism are linked with these rather than with real sex factors. Specifically, in the case in hand, if one follows this conception, copulation of the haplonts is determined by the presence or absence of two sterility factors. Oehlkers (31) points out that this interpretation is more in line with Correns's theory of sex than Kniep's original conception. He is inclined, however, to use the phrase "copulation factors," but is opposed to the genetic formulations advanced by Kniep.⁴

The position of Vandendries (45) is not quite clear. In one instance, in contrasting Brunswik's "sterility factors" and Kniep's use of the concept "fertilizing factors," he states that in the critique of his experiments he has always adopted by pure opportunism Kniep's interpretation. In the same paper he states that both these theories lie in the realm of pure hypothesis; that with respect to the law of fertility between geographic races the theory of Kniep must admit an indeterminate number of determiners, whereas the theory of Brunswik permits consideration of the loss of a single determiner as a cause of exceptionally increased fertility; and that he would be inclined to adopt the point of view of Brunswik.

⁴ This statement is based on the review by Kniep (24) of Vandendries's paper (44), which the present writer has not seen.

Further understanding of the fundamental causes operative in the production of more than two types of haplonts is dependent upon more detailed study of the phenomenon than was attempted in the work presented here. For this reason, the interpretation of results in this paper is based upon conceptions and conclusions—although not accepted in toto—that other workers have derived from more extensive investigations.

It has been assumed that the four types of haplonts are the result of segregation of factors carried by the fusion nucleus of the diplont. The fusion nuclei are said to contain two allelomorphic pairs of genes that determine the sexual nature of the individual haplonts. The four genes are thought to be borne separately on four chromosomes. Thus the genotypic formula for the fusion nucleus is written $AaBb$. It is seen that four genotypically different types of nuclei may follow chromosome segregation. The four possible genotypes would be AB , aB , Ab , and ab . Kniep postulated that crosses occur only between haplonts that have no duplication of genes. Thus the genotype Ab would be compatible with aB but not with AB or ab . The symbols used in Table 2 indicate genotypic differences in the four types of haplonts used in the present pairings. Thus it is seen that the haplonts designated as ab produced clamps when paired with all the haplonts under the symbols AB , but not with any of the other three types. Further analysis of the table shows that each group of haplonts behaved in a similar manner; that is, compatibility was found only between haplonts designated by opposite genetic formula.

Experiments by several investigators (2, 17, 19, 20, 27, 28, 39) have demonstrated that four genotypes are consistently present in the sporocarps, or hymenia, of 15 species of Hymenomycetes. Studies of the spores from individual basidia showed that some basidia bear two types of spores (21) and that other basidia bear four types of spores (16, 30). This behavior is attributed to variation in the time of the reduction division. Inasmuch as the basidia are four-spored, two divisions must follow the fusion of the dicaryon in the basidium. If the first of the divisions is the reduction division, only two genotypes are possible for each basidium, and two basidia are necessary to produce the four genotypes in one fruiting body. The nuclei resulting from the second division would be genotypically identical with their sister nuclei. If, however, the reduction division occurred in the second division, four genotypes could be borne on the same basidium, as the two daughter nuclei of the fusion nucleus would each contain the four genes $AaBb$. Therefore, if reduction division in one of the daughter nuclei gave rise to genotypes having the formulas AB and ab , and the other nucleus produced genotypes represented by the formulas Ab and aB , spores of four genotypes would be produced on one basidium. In the present work no effort was made to obtain spores from individual basidia. The spores, as stated, were transferred from an arachnoid hymenium.

INTERCROSSING OF FALSE-ANTHRACNOSE CULTURES

Table 3 gives the results of a checkerboard cross of six false-anthracnose cultures, all obtained from apples grown in Washington, and a seventh culture (No. 279-A) isolated from a New York Baldwin. Laboratory studies had shown no difference in morphology or path-

ogenicity between the western strains and this eastern culture. The numbers used are the stock numbers under which the cultures have been carried in the laboratory. The technic employed was the same as that described for the preceding experiments. Examination of the cultures was made when the hyphae of the two strains had mingled thoroughly. As shown by Table 3, clamps were found in but one pairing, that between culture No. 266-A, isolated from Rome apples shipped from Benton City, Wash., and sent to the Chicago office from Detroit, and culture No. 274, isolated from Winesap apples shipped from Wenatchee, Wash., and inspected on the Chicago market.

TABLE 3.—*Results of all possible pairings of seven so-called false-anthracnose strains*

[— indicates absence of clamps; + indicates presence of clamps]

Culture	No 145	No. 226	No 248A	No 266A	No 273	No 274	No 279A
No. 145..	—	—	—	—	—	—	—
No 226	—	—	—	—	—	—	—
No 248-A	—	—	—	—	—	—	—
No 266 A	—	—	—	—	—	+	—
No 273.	—	—	—	—	—	—	—
No 274	—	—	—	+	—	—	—
No 279-A	—	—	—	—	—	—	—

The nodose septate form resulting from the cross fruited and was studied in the laboratory. It agreed in all morphological details, including size and form of hyphae, basidia, and spores (fig. 1, G), with both eastern and western perfect forms isolated from apples showing fisheye rot. Checks were made as usual, and examination showed all checks to be free from clamps.

The cross between Nos. 266-A and 274 was repeated five times. Each time clamps were produced by the mating, and check cultures remained negative. Duplication of the balance of the checkerboard gave identical results a second and a third time. None of the other cultures produced clamps when paired. Their failure to cross will be discussed later.

CROSSING OF FALSE ANTHRACNOSE WITH FISHEYE CULTURES

These 6 western cultures of false anthracnose were representative of about 50 sterile strains isolated from western apples during the last three years. The other strains died in the laboratory. The character of the lesions from which they were isolated, as well as the morphology and consistent sterility of the mycelium, are evidence that the causal organism of false anthracnose and these sterile strains are the same fungus. Although about 20 isolations had been made from lesions on apples grown in New York identical with these western lesions, culture No. 279-A was the only strain that had remained alive during the entire period of these studies. This lack of vitality under laboratory conditions, together with the identity in morphology and pathogenicity, indicates that the eastern and western sterile strains are the same organism. The fact that No. 279-A was isolated from an apple from a second lesion of which was isolated the perfect form producing fisheye rot, together with the production by crossing of two western strains of a perfect fungus identical morphologically and pathogen-

ically with the fisheye-rot organism, would seem conclusive evidence that the sterile strains producing false anthracnose and the sterile strains of the fisheye-rot organism are identical. Inoculation studies with the four strains of the fisheye-rot organism proved that these strains are equally pathogenic. Lesions produced by the perfect strain and by the four sterile strains produced by germination of its single spores are indistinguishable. These lesions are also indistinguishable from those produced by inoculation with each of the sterile strains from false-anthracnose lesions.

In order to check further the identity of these sterile strains, pairings were made with each of these seven strains and four single-spore strains from culture No. 211-C—Nos. 1, 2, 14, and 17. (Table 2.) The results are shown in Table 4.

TABLE 4.—Results of all possible pairings of the so-called false-anthracnose strains with four genotypic strains of culture No. 211-C

[— indicates absence of clamps, + indicates presence of clamps]

Culture	No 1	No 2	No 14	No 17	No 266-A	No 274	No 145	No. 226	No 248-A	No 273	No 279 A
No 1	—	—	—	+	+	+	—	—	—	—	—
No 2	—	—	+	—	+	+	—	—	—	—	—
No 14	—	+	—	—	+	+	—	—	—	—	—
No 17	+	—	—	—	+	+	—	—	—	—	—
No 266-A	+	+	+	+	—	+	—	—	—	—	—
No 274	+	+	+	+	+	—	—	—	—	—	—
No 145	—	—	—	—	—	—	—	—	—	—	—
No 226	—	—	—	—	—	—	—	—	—	—	—
No 248 A	—	—	—	—	—	—	—	—	—	—	—
No 273	—	—	—	—	—	—	—	—	—	—	—
No 279 A	—	—	—	—	—	—	—	—	—	—	—

Because of the significance of these pairings they were made many times. It is seen that the single-spore cultures (Nos. 1, 2, 14, 17) maintained their previously noted behavior toward one another. Moreover, since previous pairings between the two western strains (Nos. 266-A and 274) had resulted in clamp formation as well as fruiting, positive results from the pairing of these two strains, obtained in this series, may be regarded as duplications of former findings.

Table 4 shows also that four of the western strains and the eastern strain did not form clamps with any of the strains with which they were paired. Although these five sterile strains have been paired in all possible combinations with each other and with the four types of haplonts from single-spore cultures six times, no clamp connections have ever been found in examinations of the pairings. This consistent maintenance of the haplophase necessitates the consideration of three possibilities. The first is the phylogenetic relationship involved. If the five sterile strains were too distantly related to the others with which they were incompatible, that is, different species, the negative results obtained would be expected. The negative results from interpairing the five variants would then indicate either that they were too distantly related to one another or that they were genotypically alike. However, cultural studies in the laboratory have disclosed no morphological, physiological, or pathogenic differences among these five sterile strains and any other haplonts studied. It is believed that the similarities noted render untenable

the hypothesis that a too distant relationship is responsible for the negative results.

The second possibility is that all the haplonts found belong to the same species, but that the affinities for these five sterile strains, although present in the progeny of the New York strain, have not been found. However, the clear-cut dihybrid behavior of the 30 single-spore cultures used in the previous work is rather strong evidence that only four genotypes are formed by this strain. Additional evidence that there are but four genotypes is provided by the following fact: No deviation from the dihybrid behavior was found when 30 single-spore cultures made from the F_1 generation were paired with each of the four genotypes that had been segregated from the parent generation. Each of the 30 haplonts formed clamps with some one of the original four genotypes. This typical dihybrid behavior among 60 haplonts seems sufficient evidence that representatives of all genotypes formed by this strain have been found.

The third possibility is occasional incompatibility, such as has been found to exist in other Hymenomycetes, among haplonts derived from individuals of the same species that were separated geographically. Vandendries (45) made studies of individuals of *Coprinus micaceus* from Luxemburg, eastern and western Canada, the Netherlands, Germany, and other widely separated localities. The behavior of the haplonts from these individuals was very erratic. Kniep (23) in reviewing this work stated that it furnishes proof that haplonts of the same species of different geographical origin can be sterile toward one another on an extensive scale. The erratic behavior among haplonts of individuals from different localities has been found by other workers in many species of Hymenomycetes and is attributed either to gene changes or to environmental factors that modify the development of the inherent genotypic tendencies. In the present work it is felt that the consistent incompatibility of the five haplonts discussed may be considered as an additional instance of such a phenomenon. Therefore, they are here regarded as haplonts of the same species even though they do not form clamps with any of the haplonts so far found in the species. This phenomenon will be more fully considered in the discussions that follow.

A further study of Table 4 shows that two of the strains (Nos. 266-A and 274) from western apples mated with one another and that each formed clamps with each of the four single-spore cultures (Nos. 1, 2, 14, 17), which represent the four types of haplonts from the New York culture No. 211-C. These results can not be interpreted in the light of the purely qualitative genetic conceptions thus far used in this paper. The fundamental premises previously used in this discussion are that duplication of the genes involved renders haplonts incompatible and that genotypic opposites are compatible. The theoretical genetic formula of the fusion nucleus previously presented, $AaBb$, permits but four genotypes. The assigning of the genetic formulas, which follow, is based on previous crossings of the haplonts. (Tables 2 to 4.) The assignment of the notation aB to strain No. 266-A and of Ab to strain No. 274 indicates that they are genetic opposites and should mate. The finding of clamps in each of the six pairings that have been made between these two is seen to be in accord with the notation. The qualitative genetic notation is

supported also by the behavior of cultures Nos. 1, 17, 14, and 2, which have, respectively, the genetic notations *AB*, *ab*, *Ab*, and *aB* when crossed among themselves (Tables 2 and 4), in that the presence or absence of clamps has been consistent with the genotypic symbols assigned to the paired haplonts.

In additional studies cultures Nos. 274 and 266-A were each paired with each of the above-mentioned single-spore cultures in two Petri dishes on string-bean agar. Microscopic study showed that clamp formation had again occurred in each of the eight pairings. Following these qualitative results a quantitative study was made. An extensive series of mounts of the mycelia was made from each of the pairings in the two plates and studied microscopically. A sufficient number of systematic studies was made to permit the mapping of the various conditions of the different areas within the traced outlines of the colonies. The results are shown in Figure 2.

An analysis of the diagrams shows that not only were the results of mating qualitative, as indicated by the presence or absence of clamps, but quantitative as indicated by the number and completeness of clamps present in each pairing. Ordinarily when two haplonts mate, normal clamps occur and oidia are absent at the point of contact of the two haploid mycelia, and the condition is reversed in both haplonts a short distance from the point of contact. In pairings held in the laboratory for some weeks it has been found that, as time passed, a decrease in haploid hyphae containing septa and producing oidia was accompanied by an increase in diploid hyphae that formed clamps and did not produce oidia. In an examination of positive pairings 3 to 4 weeks old it was found that ordinary septa were very rare and that clamps were present in abundance, while oidia were no longer found. These findings indicate very strongly that when haplonts copulate a progressive decrease of the haplophase is accompanied by a progressive increase of the diplophase. In the present case, however, no such consistent regularity of progression was found, but degrees of completeness of copulation were noted, the quantitative conception of copulation being based on differences in the included pairings and in the different areas in each pairing. Such differences involved the presence of oidia and ordinary septa (fig. 1, A), which were accepted as evidence of haploid hyphae, and the relative normality and number of clamp connections, which were considered proof of diploid hyphae. Clamps that were incomplete or variants (fig. 1, B) were considered to indicate a lesser degree of compatibility than complete normal clamps (fig. 1, C). The accompanying genetic interpretation (Table 5) of some of the crosses shown in Table 4 is included here to facilitate the discussion of these quantitative phenomena.

The genetic formulas used are those already assigned to the different strains. The plus and minus signs, as in other tables, indicate the presence or absence of clamp connections. The pairings are numbered consecutively for more ready reference.

An examination of Table 5 shows that clamps were formed in each instance in which the individual contributions of the two haplonts resulted in the production of the genetic formula *AaBb* that has been considered normal for the fusion nucleus. This is seen in pairings Nos. 2, 5, 7, 10, 18, 20, 23, 25, 28, and 33. Each of these crosses has been strong; that is, it has produced many normal clamps. Nos.

2 and 7 are duplicate crosses of Nos. 266-A and 274, which have always given strong results. Three pairings that were held in the laboratory fruited copiously. Nos. 18 and 33 are duplicate pairings of Nos. 1 and 17 and Nos. 23 and 28 are duplicates of the cross between Nos. 2 and 14, which have repeatedly given strong, positive results with copious clamp formation and sporulation.

TABLE 5.--Genetic notation of the pairings of two western and four eastern haplonts

Haplonts		No. 266-A			No. 274			No. 1		
No	Genetic notation	Pairing No	Clamps present (+) or absent (-)	Genetic notation	Pairing No.	Clamps present (+) or absent (-)	Genetic notation	Pairing No	Clamps present (+) or absent (-)	Genetic notation
266-A	<i>aB</i>	1	+	<i>aBaB</i>	2	+	<i>AbBaB</i>	3	+	<i>ABaB</i>
274	<i>Ab</i>	7	+	<i>aBAb</i>	8	+	<i>AbAb</i>	9	+	<i>ABAb</i>
1	<i>AB</i>	13	+	<i>aB AB</i>	14	+	<i>AbAB</i>	15	+	<i>ABAB</i>
2	<i>aB</i>	19	+	<i>aBaB</i>	20	+	<i>AbBaB</i>	21	+	<i>ABaB</i>
14	<i>Ab</i>	25	+	<i>aBAb</i>	26	+	<i>AbAb</i>	27	+	<i>ABAb</i>
17	<i>ab</i>	31	+	<i>aBab</i>	32	+	<i>Abab</i>	33	+	<i>ABab</i>
		No. 2			No. 14			No. 17		
266-A	<i>aB</i>	4	+	<i>aBaB</i>	5	+	<i>AbBaB</i>	6	+	<i>ABaB</i>
274	<i>Ab</i>	10	+	<i>aBAb</i>	11	+	<i>AbAb</i>	12	+	<i>ABAb</i>
1	<i>AB</i>	16	+	<i>aB AB</i>	17	+	<i>AbAB</i>	18	+	<i>ABAB</i>
2	<i>aB</i>	22	+	<i>aBaB</i>	23	+	<i>AbBaB</i>	24	+	<i>ABaB</i>
14	<i>Ab</i>	28	+	<i>aBAb</i>	29	+	<i>AbAb</i>	30	+	<i>ABAb</i>
17	<i>ab</i>	34	+	<i>aBab</i>	35	+	<i>Abab</i>	36	+	<i>ABab</i>

The remaining pairings that resulted in clamp formation, Nos. 3, 4, 6, 9, 11, 12, 13, 14, 19, 26, 31, and 32 of Table 5, really represent but six different crosses because of the duplication in the checker-board. Examination of the table shows that although clamps were formed in each instance, the theoretical formulas for the resulting fusion nuclei deviate from the assumed *ABb* normal. It is also seen that degrees of deviation occur.

For convenience the numerical designation of the crosses in the genetic Table 5 is duplicated in the diagrammatic sketch of the Petri dishes. (Fig. 2.) Comparison of the figures in relation to the crosses thus far discussed discloses a quantitative correlation. Thus cross No. 5 is the strongest experimentally (fig. 2, A) and is the only one of the four crosses between 266-A and the four genotypes that has a normal genetic formula. Cross No. 4 in Table 5 is seen to show complete duplication in both pairs of allelomorphs, and reference to the diagram (fig. 2, A) shows it to be the weakest cross. Crosses Nos. 3 and 6 each show duplication in one of the included allelomorph pairs and are both seen to be approximately midway in strength of crossing between the other two. The slight difference between crosses Nos. 3 and 6, shown in the figure, might be due to the relative importance of the genes involved or to the relative vigor of the two genotypes. Thus it is seen that the quantitative aspects of the theoretical genetic conceptions are supported by the experimental determinations.

It is believed that the sequence of the experimental findings and the theoretical applications are worthy of note. The microscopic

studies and mapping were completed before the genetic interpretations were attempted. An examination of the second diagram (fig. 2, B) reveals a complementary situation in the crosses between strain No. 274 and strains Nos. 1, 2, 14, and 17. As stated previously, strain No. 274 has repeatedly proved to be the genotypic opposite of strain No. 266-A. Therefore, opposite behavior would be expected. Comparison of crosses Nos. 10, 11, 9, and 12 in the diagram shows that the expectations are fulfilled. It is seen that in these four crosses the strongest one occurs between strain No. 274 and strain No. 2 (cross No. 10), whereas strain No. 2 (fig. 2, A) formed the weakest cross with strain No. 266-A (cross No. 4). Inasmuch as strains Nos. 2 and 14 are affinities and strains Nos. 266-A and 274 are also genotypic opposites, this behavior is in accord with expectations. Further analysis of the diagram shows that strains Nos. 1 and 17 occupy a midway position in strength of crossing with strain No. 274 (crosses Nos. 9 and 12). This is consistent with their behavior with strain No. 266-A (crosses Nos. 3 and 6). (Fig. 2, A.) To complete the consideration of the complementary relationship between strains Nos. 266-A and 274, attention is directed to cross No. 11 between strain No. 274 and strain No. 14. It is seen that the pairing of No. 14 with No. 274 resulted in the weakest cross.

A consideration confined to the relationships between strains Nos. 274 and 2 and between Nos. 266-A and 14 is in accord with a purely qualitative genetic interpretation involving two pairs of allelomorphs. The crossing of strains Nos. 266-A and 274 with strains Nos. 1 and 17, and of No. 266-A with No. 2, and of No. 274 with No. 14, can not be explained by this hypothesis. Reference to Table 5 shows that identical genetic formulas have been assigned to both positive and negative pairings, as in crosses Nos. 13 and 16. Cross No. 16 shows agreement in but one pair of genes and is negative, whereas cross No. 11 shows agreement in both pairs of genes and is positive.

DISCUSSION OF CROSSINGS

In the present work no purely genetic studies, such as interpairing or back crossing of hybrids, have been made. The results of such studies with other Hymenomycetes by Kniep (22, 23) support the contention that gene changes are involved. In view of this fact, the first question that presents itself is the correctness of the distinction between positive and negative pairings. The presence or absence of clamps is, as has been said, the accepted criterion. In the work presented in this paper efforts have been made to confirm diagnoses based on the presence or absence of clamps by subsequent presence or absence of sporulation in the cultures. Fruiting has occurred with some strains in seven to nine days on string-bean agar. However, as has been stated, the fruiting of the fungus is erratic. The writer has not been able to induce sporulation at will with any of the nodose septate strains studied. This has been true of all perfect forms isolated from apple as well as of the perfect forms that resulted from crosses in the laboratory. Therefore, although sporulation when found may be considered as positive evidence, the absence of fruiting can not be accepted as negative evidence. In the intercrossing of the six haplonts here considered (Nos. 266-A, 274, 1, 2, 14, and 17)

spores have been found, as stated in different places herein, in the crosses between Nos. 266-A and 274, Nos. 1 and 17, and Nos. 2 and 14, but not as yet in any of the other crosses.

The formation of clamps that has occurred in the pairings between the eastern and western haplonts and the agreement in morphology and pathogenicity seem ample proof that they belong to one and the same species. Hanna (17), working with geographical strains of *Coprinus lagopus* from England and from Canada, found that haplonts from the two geographical strains reacted positively. She stated:

The fact that hyphal fusions took place between the English and Canadian strains, resulting in the formation of clamp-connexions, furnishes a conclusive proof that the *Coprinus lagopus* found in England is identical with the species occurring in Canada.

Vandendries (41) reported that he had crossed *Panaeolus campanulatus* and *P. fimicolo*, but both Kniep (22) and Brunswik (3) state that all efforts to cross species have failed and that this cross had not been repeated and is questionable.

The complete fertility recorded in Table 5 between each of the two western haplonts and the four genotypes from the eastern strains is in accord with the findings of other workers. For example, Hanna (17) found that the individual fruit bodies of *Coprinus lagopus* consistently produced four genotypes. That is, the testing of the haplonts resulted in typical dihybrid results such as are recorded in Table 2 in this paper for *Corticium centrifugum*. However, when she paired any haplont from a fruit body of one locality with the four genotypes from a fruit body from another locality, each pairing was positive. In 694 pairings that were made between haplonts from different fruit bodies she found that while each fruit body produced but 4 "sexes" the 6 fruit bodies in the aggregate produced 24 "sexes." She stated:

Further investigations may show that these sexual strains are to be found generally throughout the heterothallic Basidiomycetes, and that each species is made up of a definite number of such strains.

Other instances of haplonts showing a typical monohybrid or dihybrid type of behavior when paired with other haplonts from the same fruiting body but copulating with all the haplonts from different fruiting bodies of the same species, obtained from slightly or widely separated localities, have been reported by Kniep (21, 22, 23) for *Schizophyllum commune*, *Aleurodiscus polygonius*, *Collybia velutipes*, *C. conigena*, *Armillaria mucida*, and *Coprinus fimetarius*; by Brunswik (2, 3, 4) for *C. fimetarius*, *C. comatus*, *C. niveus*, *C. picaceus*, *C. lagopus*, and *C. friesii*; by Vandendries for *Panaeolus campanulatus* (41) and for *C. radians* (42); and by Newton (29) for *C. rostrupianus*.

Taking into consideration the results of these workers, it would seem that the two western haplonts studied in this paper represent two of four genotypes and that the other two genotypes have not yet been found. A brief résumé and correlation of the presentation in this paper with the findings of other workers follows.

The phenomena reported here for *Corticium centrifugum*, that is, the dihybrid behavior recorded in Table 2, the interincompatibility and intraincompatibility of five haplonts, as shown in Tables 3 and 4, and the apparent quantitative manifestation illustrated in the diagrams (fig. 2), are of the same order as those that have been reported

for other Hymenomycetes. A comprehensive discussion of the subject will be found in papers by Kniep (23) and Brunswik (5).

Many of the Hymenomycetes, which are dioecious (heterothallic), have been shown, by crossing of the haplonts from individual fruiting bodies, to follow the clear-cut dihybrid ("tetrapolar" or "viererschema") type of behavior recorded for *Corticium centrifugum* in Table 2. A smaller number have shown a monohybrid ("bipolar" or "zweierschema") type of behavior, of which there is no indication in *C. centrifugum*.

In addition to the previously discussed deviation from the "tetrapolar" condition that occurs when haplonts from different fruiting bodies are paired, deviations have been found to occur in the relationship of haplonts from the same fruiting body. Reports of deviations from either the "bipolar" or "tetrapolar" type of behavior in the haplonts from a single fruiting body have been made by Vandendries (41, 44, 45) for *Panaeolus campanulatus*, *P. separatus*, and *Coprinus micaceus*; by Kniep (22) for *Schizophyllum commune*; and by Brunswik (4) for *C. picaceus*. However, enough consistent regularity was found to indicate that merely a deviation from the type is involved rather than an entirely different phenomenon. Brunswik (4) reported for *C. picaceus* a 25 per cent deviation from the typical dihybrid behavior and termed the phenomenon "Durchbreckungskopulation."

All the haplonts of *Corticium centrifugum*, when carried in the laboratory in pure culture, have maintained their sterile condition. Some of the strains have been in culture for nearly three years, but no clamp formation or fruiting has been observed. Other workers have found a spontaneous changing from the haplophase to the diplophase. Vandendries (42) terms this phenomenon "l'hétéro-homothalisme." This phenomenon has been reported by Kniep (20) for *Schizophyllum commune* and *Collybia velutipes*; by Vandendries (42) for *Coprinus radians* and *C. micaceus*; by Hanna (17) for *C. lagopus*; and by Newton (29) for *C. rostrupianus*.

It is seen that investigations of dioecious Hymenomycetes thus far have indicated that either a monohybrid or dihybrid behavior is consistent enough to be considered typical or normal. It is also clear that deviations of different kinds occur. Kniep (22, 23), in maintaining his fundamental assumption that presence of like genes in any two haplonts prevents copulation and that genotypic opposites do copulate, assumed that gene changes cause these deviations. He contended that no completely different genes are involved, but that the genes are localized in homologous chromosomes in the same position, but are slightly changed quantitatively. In other words, the phenomenon of multiple allelomorphs is involved. Thus, the genetic formula of the fusion nucleus may change from $AaBb$ to $A'a'B'b'$, or the change may affect but one pair of allelomorphs. The haplonts containing the modified genes maintain the dihybrid ratio between each other, but each will copulate with any haplont containing unmodified genes. The disruption of the typical monohybrid or dihybrid ratios among haplonts from the same fruiting body, previously referred to, and the complete interfertility of haplonts from different localities are attributed to quantitative gene changes.

Cases of complete intersterility are also reported. Kniep (23) has reported that a haplont of *Schizophyllum commune* did not copulate with any of the haplonts from another fruiting body. Vandendries

(42) has reported a similar phenomenon for *Coprinus radians*. Brunswik (4) found the same situation in *C. comatus*. The most striking example is that reported by Vandendries (45) for *C. micaceus*. Interpairing of haplonts from different geographical strains showed two types of deviations. Some of the haplonts were incompatible, while others showed increased compatibility. It is believed that the incompatibility in the case of the five haplonts discussed in this paper and the complete compatibility of the other two western haplonts comprise an analogous situation. Kniep (23) reported that the haplonts of two fruiting bodies of *Collybia cirrhata* collected in one locality were completely interfertile, but that haplonts from two other fruiting bodies collected in different places in another locality were absolutely intersterile. The completely interfertile forms are easily explained by the previously advanced theory of quantitatively altered genes. However, the complete intersterility of the two other fruiting bodies can not be explained by the complementary assumption of identity of genes in all eight component haplonts, for since the four haplonts of each fruiting body follow a pure or slightly modified dihybrid formula they must be different genotypically. Kniep considers that the intersterility may be due to too great a quantitative difference in the genes or to secondary factors.

The spontaneous change of mycelia from the haplophase to the diplophase is also assumed by Kniep (23) to be the result of gene changes. In a monohybrid type the change involves one allelomorphic pair while in a dihybrid two pairs of genes are modified.

The genetic interpretation of the behavior of the Hymenomycetes advocated by Kniep (21, 22, 23) thus embraces the conception that the forms are highly mutable. This interpretation finds support in extensive purely genetic studies by Kniep and other workers. Kniep (23), however, states that he has at present no satisfactory explanation for all the deviations that have occurred in his tests of *Schizophyllum commune*, and in those of Vandendries (45) with *Coprinus micaceus*, and in those of Brunswik (2) with *C. fimetarius*. Possibly they may necessitate an entirely different interpretation. At present the genotypic explanation serves better than any other as a working hypothesis.

In the light of this brief consideration of the literature, it is seen that the results of the study of the apple pathogene presented in this paper are in accord with the findings of other workers for numerous Hymenomycetes. No deviations have been found in the studies of the apple organism that have not been reported as occurring within a single species of other Hymenomycetes.

TAXONOMY

Since the fungus described in this paper compares so closely in pathogenicity and morphology with the one described by Eustace as *Hypochnus* sp., there is little doubt that it is the same fungus. Taxonomic studies carried out in the present work have shown that, although much attention has been given to the group, a great deal of confusion still exists in regard to the taxonomy of *Hypochnus* and closely related genera.

The genus *Hypochnus* was described by Fries (14) in 1818. In 1874 (15), however, he placed *Hypochnus* as a subgenus under *Cor-*

tium, reserving for *Hypochnus* those species of *Corticium* which possessed a floccose or tomentose to almost powdery hymenium. In 1881 the genus *Hypochnus* was amended by Kärsten (18), who included in it most of the congeneric forms originally included by Fries, but excluded such as fell logically into the genus *Corticium*. *Hypochnus* as amended by Kärsten includes the synonymous genus *Tomentella* ascribed to Persoon (33, v. 2, p. 18-19) but first published as a genus by Patouillard (32) in 1887. The genus *Hypochnus* as given by Saccardo (35) is a composite group based on the old Fries classification but includes forms which Fries had later discarded from the genus. Many species described by Saccardo under *Hypochnus* are now placed in *Corticium*, *Peniophora*, and related genera. Engler and Prantl (12) and Stevens (37) separate the genus *Hypochnus* Ehrenberg (11) from closely related genera on the basis of spore color and number of sterigmata, and following the suggestion of Schroeter (36), place it in the family *Hypochnaceae*, which is separated from the *Thelephoraceae* on the basis of the absence in the former group of a compact layer of closely aggregated basidia forming a true hymenium. H. and P. Sydow (38) advise the dropping of *Hypochnus*, in the sense of Schroeter, and the assignment of the species to their proper places in other genera. Burt (7) agrees that *Hypochnus*, in the sense of Schroeter, must be abandoned, and follows Kärsten in placing under *Hypochnus* only resupinate species with colored echinulate spores. Resupinate forms with smooth hyaline spores, placed by Engler in *Hypochnus*, would, therefore, be excluded. Burt (8) assigns to the genus *Corticium* those species, both hypochnoid and compact, which are always resupinate, have colorless spores, and lack cystidia, excepting a few parasitic forms placed in *Exobasidium*.

The fungus under discussion fits closely Burt's description of *Corticium centrifugum* (Lev.) Bres., which is synonymous with the *Hypochnus centrifugus* Lev. described by Saccardo (35, v. 6, p. 654). Burt (8) stated, however, that *C. centrifugum* is "only rarely nodose septate." Lindau and Ulbrich (26) in their description of *C. centrifugum* say that clamp connections are abundantly present. It is highly probable that the heterothallic nature of the fungus is responsible for these contradictory statements. As clamp connections are not formed in the sterile strains and are consistently present in the perfect strains, some variation is to be expected. In material in which fusion had recently occurred clamp connections would be present in some hyphae and not in others. (Fig. 1, D, and Fig. 2.) Therefore, the number of clamp connections found in material collected in the field would be proportional to the relative proportion of sterile and perfect mycelium present in the specimen. Examinations of crosses made with compatible strains of this fungus in the laboratory have shown great variations in the number of clamp connections present. Soon after crossing takes place clamps occur only near the point of contact, while the rest of the colony growth is made up of hyphae with ordinary septa and oidia. (Fig. 2.) Later the new growth at the periphery of the colonies shows perfect clamps (fig. 1, C; fig. 2, A, 14, and B, 2), and in the case of strong crosses some imperfect or incomplete clamps (fig. 1, B) even develop in the old growth near and over the inoculum (fig. 2, A, 14, and B, 2.) When the culture is about 2 weeks old clamps are the rule and ordinary septa the exception.

Corticium centrifugum is rather widespread in the United States and has been reported from three of the States (New York, Washington, and Oregon) from which the apple-rotting fungus has been collected. This fact further substantiates the relegation of the apple fungus to this species.

DESCRIPTION OF CORTICIUM CENTRIFUGUM FROM PURE-CULTURE STUDY

Mycelium spreading over the substrate and forming a delicate white mat, varying from closely appressed to fluffy and occasionally zoned; center of mat thicker than extremities; older cultures on bean pods or apple twigs forming hyphal knots, or plectenchymic cushions 3 to 4 mm. in diameter, white when young, brownish (cinnamon buff to avellaneous)⁶ when old; hyphae smooth, white, loosely interwoven, not incrusting, diameter variable, averaging 3.5 μ , dihybrid; clamp connections characteristically present in perfect forms (fig. 1, C, E), absent in sterile strains (fig. 1, A); hymenium arachnoid, powdery, indefinite; basidia borne directly on hyphae (fig. 1, F, G), clavate 3.5 to 5.5 by 9 to 20 μ , bearing four sterigmata from 1.85 to 5.5 μ (fig. 1, H); no cystidia; spores subglobose to ovate, smooth, hyaline, flattened on one side, 3.4 to 3.6 by 5.4 to 7 μ average, 2.7 by 3.7 μ minimum, 4.6 by 8.3 μ maximum (fig. 1, F, G, H). Oidia present in sterile strains. (Fig. 1, A.)

Habitat: Parasitic on apple fruit. Occurs in New York, Illinois, Washington, Idaho, Oregon, and Virginia.

SUMMARY

The diseases known as Hypochnus rot, fisheye rot, and false anthracnose are identical and are caused by the same fungus, which was previously designated as *Hypochnus* sp., but which is here identified as *Corticium centrifugum* (Lev.) Bres.

The decay in apples progresses at the ordinary commercial cold-storage temperature of 32° F. Indications are that infection occurs in the orchards and that the disease develops but does not spread in transit or storage.

The fungus has been found to grow well on artificial media. Vegetative growth is favored by high humidity and is extremely sensitive to desiccation.

Studies of the fungus have shown that it is heterothallic and that individual perfect strains produce four types of haplonts. The results of crossing studies made with sterile strains have been interpreted genetically and are in accord with the findings of other workers for other Hymenomycetes. The identity of eastern and western strains of the fungus based on morphological and pathogenic similarities has been supported by crossing of sterile strains.

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SOME CONDITIONS AFFECTING THE STORAGE OF PEPPERS¹

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INTRODUCTION

A quantity of green peppers (*Capsicum*) grown in the vicinity of Washington, D. C., and stored in a local cold-storage house at a temperature of about 0° C. from October 24 to November 20, 1924, was inspected at the request of the owner. The fruits were in various stages of deterioration and were thought to have been injured by freezing. However, a careful examination of the lesions led to the belief that the trouble was caused by a fungus rather than by freezing, (1) because the lesions were more definitely localized than is usual in freezing injury, and (2) because a septate mycelium was found in affected areas. Fifty-one isolations from lesions in all stages of development were made from the diseased peppers to carrot-agar plates, with the following results: 46 plates yielded pure cultures of a species of *Botrytis*, 4 were sterile, and 1 yielded an unidentified fungus. Typical lesions developed in pepper fruits inoculated with the *Botrytis*.

A fruit rot of pepper caused by *Botrytis cinerea* Pers. which occurred at the Missouri Botanical Garden in 1911 was reported by Peltier² in 1912. The disease was characterized as follows:

The peduncles were covered with a dark-brown mass of conidia and mycelium, which so weakened the tissues that the fruit soon fell to the ground. On opening the peppers a number of large, flat, crustlike sclerotia, 1 cm. long and 0.5 cm. wide, were found to fill the interior. In most cases the seeds were also covered with the crustlike masses.

This appears to be the only report on record of a disease of pepper caused by *Botrytis cinerea*, the fungus responsible for the storage rot.

The purpose of this paper is (1) to describe the freezing injury of peppers and the *Botrytis* disease as it develops in storage; (2) to submit data regarding the cause of the disease; (3) to report the effect of certain storage temperatures and humidities on development of the disease; and (4) to present data, obtained in connection with some of the storage tests, showing the influence of temperature and humidity on shriveling, ripening, and infection with and development of anthracnose (*Colletotrichum nigrum* E. and H.).

¹ Received for publication Jan. 15, 1930; issued September, 1930.

² PELTIER, G. L. A CONSIDERATION OF THE PHYSIOLOGY AND LIFE HISTORY OF A PARASITIC BOTRYTIS ON PEPPER AND LETTUCE. Missouri Bot. Gard. Ann. Rpt. 23. 41-74, illus. 1912.

DESCRIPTION OF FREEZING INJURY

A number of freshly gathered green peppers were frozen under controlled laboratory conditions, and a study was made of the symptoms of injury and the temperature at which the peppers freeze. It was found that freezing injury usually is not difficult to recognize. This injury is characterized by a soft, watery, flabby texture of the affected tissues (pl. 1, C), which may be restricted to a limited area in case of slight exposure or which may include the entire pepper. This is in contrast to the firm, brittle tissue of an uninjured pepper. (Pl. 1, A.) The external color of a pepper injured by freezing is darker than that of an uninjured pepper and is characterized by a dead and water-soaked appearance. The stem is usually soft and darker in color than that of an unfrozen pepper, and the epidermis shows a tendency to slough off when handled. When a frozen pepper is cut across, water quickly collects over the cut surface of the affected portion of the rind and drips off very freely if the tissue is squeezed. In addition, these surfaces are dark and water-soaked without the traces of white usually noted in the rind, in the segment partitions (septs), and in the core of the sound fruit. In cases of severe injury the seeds are brown in color instead of light green to white.

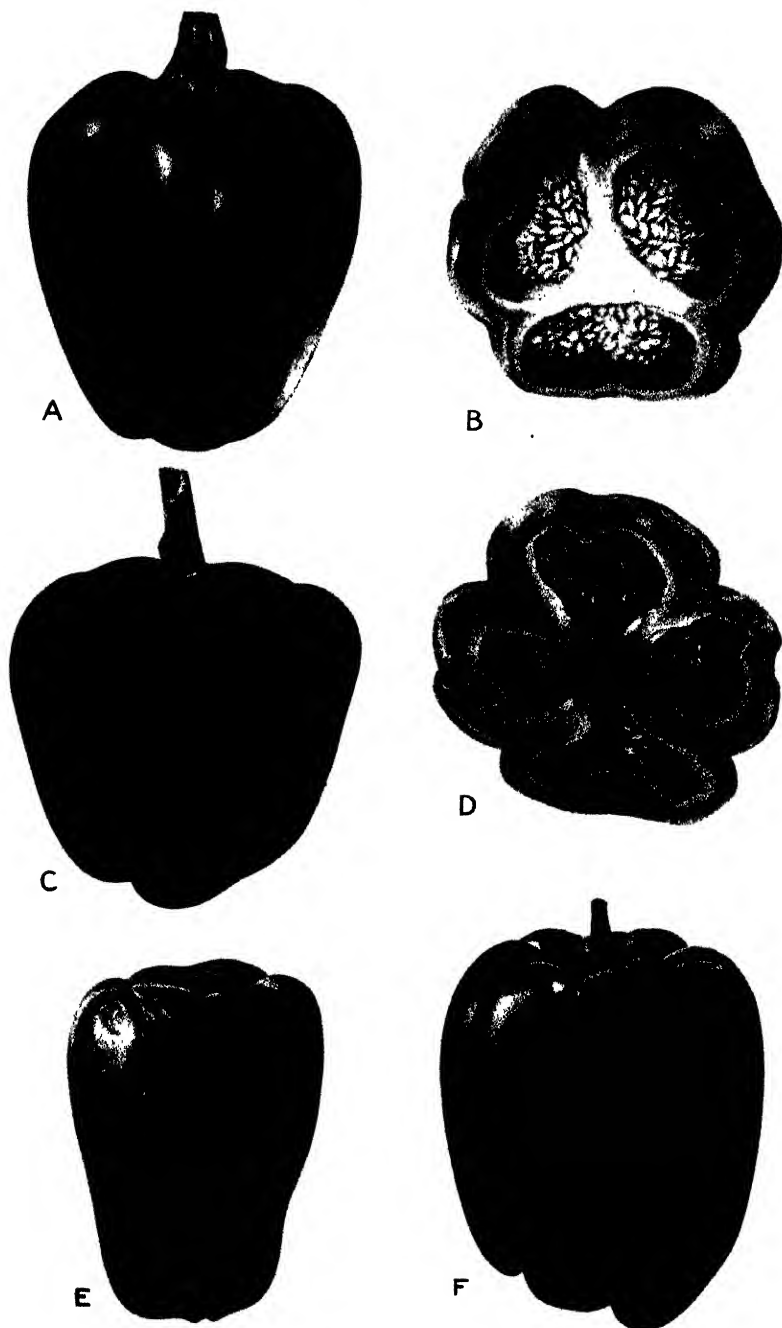
Plate 1 shows cross sections of a frozen and an uninjured pepper. In Plate 1, D, can be seen the dark color of the core and surrounding seed and to some extent the general state of collapse due to freezing injury. The white core and light-colored seed and the generally turgid appearance of an uninjured pepper are shown in Plate 1, B.

The freezing point as determined by the thermoelectric method was found to range from -1.15° to -0.99° , with an average of -1.06° C.

DESCRIPTION OF BOTRYTIS ROT

Most of the Botrytis-rot lesions were circular to elliptical, although some were irregular in outline due to the coalescence of two or more lesions. They varied in size from a mere point to an area so large that it involved most of the fruit. The color of the smaller lesions resembled a vertiver green,³ which usually persisted at the margin of the larger ones. The central areas of the larger lesions varied from Isabella color, ceru-olive, and light brownish olive to buffy olive. The color of a given lesion seemed to vary more or less in this central region, depending on the amount of light, the angle at which light struck it, and the variation in the reflected light from the glazed surface of the pepper. The epidermis in the necrotic areas remained intact and could be peeled off readily. The underlying tissue had lost its coherence and was soft and watery. Some of the lesions, especially the smaller ones, conformed to the contour of the fruit, while others were slightly sunken, with sharp margins. In many of the larger lesions the surface was wrinkled and the diseased tissue tended to collapse toward the center of the fruit. The gray mass of conidia and mycelium may sometimes be seen on the surface of the fruit when the epidermis is broken. An excellent representation of the lesions is given in Plate 1, E and F.

³ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. Pls. XXX and XLVII. Washington, D. C. 1912



LITHO EASTERN OFFSET INC. BALTO

A, Uninjured green pepper fruit; B, cross section of uninjured fruit; C, fruit injured by freezing; D, cross section of fruit injured by freezing; E, F, pepper fruits showing lesions of different sizes caused by *Botrytis cinerea*

(Colored drawings for A and C were made by Miss M. D. Arnold and for B, D, E, and F by R. C. Steadman.)

INOCULATION EXPERIMENTS

The results recorded in Table 1 were obtained from four experiments carried out to determine the pathogenicity of the *Botrytis* obtained from the foregoing isolations. In each experiment the peppers were washed with soap and water and rinsed in tap water. In experiment 1 an attempt was made to sterilize more completely the surface of the fruit by sponging with a wad of cotton saturated with 95 per cent ethyl alcohol. The peppers were inoculated by inserting a mixture of agar, mycelium, and spores from carrot-agar plates into the rind tissue by means of a sterile needle. The check fruits were merely punctured with a sterile needle. In experiments 1 and 2 a pure-line strain of *Botrytis* obtained from the original isolations was used, and in experiments 3 and 4 a reisolation from experiment 2 was employed. In each experiment both the inoculated fruits and the checks were stored under identical conditions in either moist chambers or 4-quart "till" baskets at the temperatures given in Table 1. Fifty-five infections, or 87 per cent, resulted from 63 inoculations. Only one infection occurred in the checks, and this did not occur at the point of puncture. *Botrytis* was obtained 24 times out of 26 isolations. The lesions were typical of those from which *Botrytis* was originally obtained.

TABLE 1—Results of inoculation experiments with *Botrytis* on green pepper fruits

Experiment No	Temperature of incubation	Inoculated fruits				Reisolations		Checks		
		Fruits used	Inoculations	Infections	Infection	Made	Yielding <i>Botrytis</i>	Fruits used	Needle punctures	Infections
		Number	Number	Number	Per cent	Number	Number	Number	Number	Number
1	22.25	3	7	5	71	8	8	3	5	0
2	15.5	11	11	11	100	10	8	8	8	0
3	15.5	30	30	25	83			5	5	1
4	10.0	15	15	14	93	8	8	5	5	0
Average Total		59	63	55	87	26	24	21	23	1

^a 2 isolations were made from each of 4 lesions in experiment 1. In the others each isolation was made from a separate lesion.

^b This infection occurred at a point other than the puncture.

It is believed that these results, together with those discussed later, establish the fact that the necrotic condition of the peppers described was caused by this *Botrytis*.¹

STORAGE EXPERIMENTS

DEVELOPMENT OF BOTRYTIS ROT IN DIFFERENT LOTS OF PEPPERS AT LOW TEMPERATURES

Five experiments were conducted to determine whether or not the storage of green pepper fruits at low temperature would normally result in infection by *Botrytis* in the absence of artificial inoculation. The fruits used in four of the experiments were purchased on the local

¹ H. H. Whetzel, of Cornell University, to whom the organism was submitted for identification, expressed the belief that it belonged to the *cinerea* type of *Botrytis*. The pathogene has been given the accession number 146.

market and were of unknown variety. The peppers employed in the fifth experiment were grown at the Arlington Experiment Farm, Rosslyn, Va., and were a mixture of the following varieties: Sweet-Meat Glory, Ruby King, Ruby Giant, and Bull Nose. Of the lots obtained on the market, one came from Florida, one was grown in the vicinity of Washington, D. C., and the origin of the other two is unknown.

Only sound fruits were used. They were stored in refrigerator rooms 8 feet wide, 14 feet long, and 11 feet high, in either 4-quart till baskets or 16-quart hampers. The results obtained are recorded in Table 2.

TABLE 2.—*Influence of low temperature on infection of pepper fruits by Botrytis*

Experiment No.	Where peppers were grown	Date of storage	Duration of storage	Temperature of storage	Fruits used		Infected	Lesions	Diameter of lesions	Isolations made	Times Botrytis was isolated	Other organisms isolated
			Days	°C.	Number	Number	Number	(*)	Mm.	Number	Number	
1	Not known (obtained on market, Washington, D. C.).	1924	57	-1	3	2						
		1924	57	0-2	6	6						
2	Vicinity of Washington, D. C.	1926	48	0-2	17	15	43	1-40	15	12	2	2 plates yielded <i>Penicillium</i> , and 1 an unidentified fungus.
3	Not known (obtained on market, Washington, D. C.).	1926-27	36	0-2	25	3	3	--	2	1	1	plate, sterile.
		1926-27	43	0-2	25	a 6	6	--	5	0	2	plates yielded <i>Mucor</i> sp., 1 <i>Penicillium</i> sp., and 2 bacteria.
4	Florida.....	1926-27	29	0-2	50	a 4	4	---	4	0	2	plates yielded <i>Mucor</i> sp., 1 bacterium, and 1 was sterile.
5	Arlington Experiment Farm, Rosslyn, Va.	1927	41	0-2	54	54	(b)	1-50	24	19	5	plates, sterile.

a The lesions in these 2 cases were slightly atypical, being greener than the normal.

b Numerous.

The character of the lesions that developed was typical of Botrytis rot, the percentage of the fruits infected was high, and the number of lesions in experiments 1, 2, and 5 was large. Botrytis was obtained in most of the isolations made. The lesions that developed in experiments 3 and 4 were hardly typical of Botrytis rot, being slightly greener than normal lesions. The resemblance to the normal Botrytis lesions, however, was sufficiently marked to justify making isolations from them. Only a few fruits were infected, and the number of lesions was small. Botrytis was obtained only once out of 11 isolations. Four plates yielded a *Mucor*, three showed bacteria, one a *Penicillium*, and two were sterile.

The foregoing data justify the following conclusions: (1) Different lots of peppers do not react uniformly to Botrytis infection when stored at temperatures ranging from 0° to 2° C., and (2) peppers obtained on the market at Washington, D. C., or grown locally may become infected by Botrytis when stored at low temperatures for long periods.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON INFECTION OF PEPPERS BY BOTRYTIS

Two experiments were conducted during the fall of 1928 to determine the effect of certain temperatures and humidities on the infection of pepper fruits by Botrytis during storage. Ruby King was the variety employed in both cases. It was grown at the Arlington Experiment Farm and stored in galvanized-iron chambers 40 inches high, 42 inches long, and 35 inches wide, except in two cases (the two humidities at 13° C., Table 3), where they were stored in insulated refrigerated rooms 8 by 8 by 8 feet.

There were nine galvanized chambers, three of which were located in each of three insulated refrigerated rooms 8 feet wide, 14 feet long, and 11 feet high. The temperature was automatically controlled at 0° C. in one of these rooms, at 4.5° in another, and at 10° in the third. Three humidities were maintained at each temperature by means of water and calcium chloride in evaporating pans. The low, medium, and high humidities at one temperature were fairly comparable to the low, medium, and high of the others, if the saturation deficit is used as a measure of the degree of humidity.

The temperature in the rooms maintained at 13° C. was hand-controlled by means of brine coils and electric heaters. Water and calcium chloride in evaporating pans were used to maintain the difference in humidity. Both of these humidities when measured by the saturation deficit were lower than any of the humidities employed at the other three temperatures.

In one experiment 10 full-sized green fruits were stored under each of the nine combinations of temperature and humidity at the temperatures 0°, 4.5°, and 10° C. These fruits were examined at frequent intervals for infection with Botrytis rot and anthracnose and for shriveling and ripening of the fruits.

In the second experiment fruits of four types as to size and ripeness were selected, namely, small green (half size), large green (mature green), semiripe, and ripe. Fourteen small green, ten large green, nine semiripe, and ten ripe fruits were stored in 4-quart till baskets at each of the 11 combinations of temperature and humidity given in Table 3. Frequent inspections were made for Botrytis infection, anthracnose infection, shriveling, and the degree of ripening.

TABLE 3.—Relation of temperature, humidity, and time to infection of pepper fruits by Botrytis

Temperature	Relative humidity	Fruits used	Number of fruits infected by Botrytis after—						
			11 days	18 days	25 days	32 days	39 days	46 days	53 days
° C.	Per cent	Number							
13	76	43	0	0	0	0	0		
13	71	43	0	0	0	0	0		
10	98	43	0	0	2	3	6	7	
10	89	43	0	1	1	1	3	4	
10	84	43	0	0	1	1	1		
4.5	95	43	0	2	3	6	17	20	
4.5	87	43	0	2	3	3	8	16	
4.5	74	43	0	0	1	3	5		
0	90	42	0	0	0	2	3	4	19
0	80	43	0	0	0	1	2	4	13
0	69	43	0	0	0	0	1		

The data in the two experiments, with respect to the conditions under which they were conducted, were parallel and correspond so closely that only those from the second experiment will be presented.

Although there was some variation in the number of infections by *Botrytis* occurring under given conditions at a given time in the various lots, it was not large, and the relation of infection to temperature and humidity in the different lots corresponded closely. For example, the percentage of infection under all of the 11 conditions in 39 days was in ripe fruits 8, in small green 9, in large green 12, and in semiripe 10, and the distribution at the various temperatures and humidities was about the same.

The summary of the results obtained from the four lots is recorded in Table 3. There was no infection at 13° C. in 39 days. Humidity in this case was probably a limiting factor, for infection was obtained in another experiment in a lot stored at 13° and a relative humidity of 92 per cent. It is also possible that temperature may have aided in eliminating infection, for it will be noted that the number of infections occurring at all the relative humidities at 4.5° was markedly higher than at 10°, notwithstanding the fact that the relative humidities at the two temperatures were comparable.

A larger number of infections occurred at all the relative humidities at 4.5° than at 0° C., and the initial infections were observed 14 days earlier.

At each of the temperatures 0°, 4.5°, and 10° C. the higher the humidity the larger was the number of infections, except at 0° after 46 days. Although there was a tendency for the time required for infection to occur to lengthen with the lowering of the relative humidity at a particular temperature, there were exceptions, and the difference in time was never marked.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON INFECTION OF PEPPERS WITH ANTHRACNOSE

The data on anthracnose (Table 4) were obtained in connection with study of *Botrytis* rot, but as they bring out facts of importance in regard to the storage of peppers they are included here. It should be said at the outset that very little anthracnose developed in fruits in any of the experiments aside from the last two now under consideration. In these anthracnose was more conspicuous than *Botrytis* rot, not only as manifested by the number of fruits infected but by the much larger number of lesions. Anthracnose lesions developed at all the temperatures employed, and at all the humidities except 69 per cent at 0° C., although the fruits were free from visible infection of any kind when stored. There was very little difference in the number of fruits infected at temperatures of 4.5°, 10°, and 13° at any time during the experiment. As the temperature fell to 0° there was a considerable decrease in the number of infections. There was a slight tendency for the time required for the occurrence of the initial infection to lengthen as the temperature was lowered from 13° to 4.5°. The time required for infection to occur was considerably lengthened as the temperature was lowered from 4.5 to 0°.

TABLE 4.—*Influence of temperature, humidity, and time on infection of pepper fruits with anthracnose*

Temperature	Relative humidity	Fruits used	Number of fruits infected after—					
			11 days	18 days	25 days	32 days	39 days	46 days
° C	Per cent	Number						
13	76	43	1	2	5	7	9	
13	71	43	0	1	3	4	8	
10	98	43	1	1	4	7	12	17
10	89	43	0	1	2	2	7	12
10	84	43	0	0	1	3	15	
4.5	95	43	0	1	3	7	12	21
4.5	87	43	0	0	1	5	8	20
4.5	84	43	0	1	2	9	12	
0	90	42	0	0	0	0	0	2
0	80	43	0	0	0	1	4	5
0	69	43	0	0	0	0	0	

The number of infections that occurred at 13° C. may have been limited by the low humidity employed, although there is little evidence in the table as a whole that humidity was a limiting or modifying influence. At 13° the number of infections was less at 71 per cent relative humidity than at 76 per cent. At temperatures of 4.5° and 10° the smallest number of infections occurred at the medium humidities, the greatest number fluctuating between the high and low humidities with extension of time. At 0° the number of infections was greatest at the medium humidity. The distribution of infection in the individual lots, as in the other experiment, showed the same general relation to temperature and humidity as did the four lots jointly.

It should be kept in mind that the results obtained on the distribution of infection at the various temperatures and humidities may have been influenced to some extent by the distribution of the inoculum, which was contingent upon natural inoculation rather than on a controlled distribution. It is believed, however, that considering the abundance of infection, the distribution must have been fairly uniform.

The following facts may be emphasized: (1) Peppers may become infected with anthracnose, and the disease progresses under the storage conditions employed in these experiments; (2) at a temperature of 0° C. infection is delayed sufficiently to make storage of uninfected fruits, although contaminated with anthracnose spores, feasible for a period of 32 to 39 days.

An examination of the data on the various lots of peppers revealed a marked difference in the amount of infection of small green and large green fruits on the one hand and semiripe and ripe fruits on the other. Nine per cent of the large green fruits and 9 per cent of the small green fruits stored at all the conditions of temperature and humidity became infected by the end of 39 days, while 21 per cent of the semiripe and 28 per cent of the ripe fruits became infected. Barring the possibility that this difference in infection might have resulted from a difference in the amount of inoculum due to a difference in the length of time that fruits remained on the plants, it would seem that these results indicate a greater susceptibility to anthracnose in the semiripe and ripe fruits than in the small and large green fruits.

Dastur ⁵ claims that the ripe fruits are more susceptible to anthracnose than the green ones.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON RIPENING OF PEPPERS IN STORAGE

The results on ripening are recorded in Tables 5 and 6. Table 5 deals with the small green and large green fruits, and Table 6 with the semiripe fruits. The results with the small green and the large green fruits were so similar that they were combined.

TABLE 5.—*Influence of temperature, humidity, and time on ripening of green pepper fruits in storage*

Temperature	Relative humidity	Number of fruits showing indicated degree of ripening after—															
		Fruits	11 days			18 days			25 days			32 days			39 days		
			Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe
° C.	Per cent	No															
13	76	24	1	5	0	2	2	4	4	2	5	4	6	5	2	8	6
13	71	24	2	3	0	5	2	3	3	5	4	2	9	4	2	10	4
10	98	24	4	0	0	2	6	0	3	7	1	3	7	2	3	7	3
10	89	24	1	2	0	2	6	0	3	7	1	4	7	2	4	9	2
10	84	24	0	8	0	5	5	4	3	7	5	4	6	7	2	9	7
4.5	95	24	0	0	0	0	0	0	1	0	0	2	0	0	3	0	0
4.5	87	24	2	0	0	3	0	0	2	0	0	4	0	0	1	3	0
4.5	74	24	0	0	0	0	0	0	2	0	0	4	0	0	4	0	0
0	90	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	80	24	1	0	0	1	0	0	1	0	0	2	0	0	2	0	0
0	69	2.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6.—*Influence of temperature, humidity, and time on ripening of semiripe pepper fruits during storage*

Temperature	Relative humidity	Fruits	Number of fruits becoming ripe after—				
			11 days	18 days	25 days	32 days	39 days
		Number					
° C.	Per cent						
13	76	9	7	8	9	—	—
13	71	9	2	8	8	9	—
10	98	9	7	8	8	9	—
10	89	9	6	7	7	7	7
10	84	9	5	8	8	9	—
4.5	95	9	0	3	3	4	4
4.5	87	9	1	2	3	2	4
4.5	74	9	0	0	0	0	1
0	90	9	0	0	0	0	0
0	80	9	0	1	1	1	1
0	69	9	0	0	0	0	5

The humidities employed in these experiments seem to have had little or no effect on ripening.

The ripening process went on at all four temperatures (0°, 4.5°, 10°, and 13° C.), but very slowly at 0°, and the rate increased rapidly with the rise in temperature. However, ripening was sufficiently slow in green peppers at both 0° and 4.5° to permit storage for three or four weeks.

⁵ DASTUR, J. F. *GLOMERELLA CINGULATA* (STONEMAN) SPAULD. AND V. SCH. AND ITS CONIDIAL FORMS, *GLEOSPORIUM PIPERATUM* E. AND E. AND *COLLETOTRICHUM NIGRUM* E. AND HALS., ON CHILLIES AND CARICA PAPAYA. *Ann. Appl. Biol.* 6: 245-268, illus. 1920.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON SHRIVELING OF PEPPERS IN STORAGE

At the first sign of softening a pepper fruit was considered shriveled. It was believed that this procedure would result in a more accurate measure of shriveling than any attempt to estimate the degree of shriveling. Since the physical condition of the fruit is more important from the market standpoint than the loss in weight, shriveling rather than loss in weight was used as a measure of loss of water.

With one exception the results, whether considered in connection with the separate lots or jointly, clearly show a marked effect of both temperature and humidity on shriveling. The higher the temperature and the lower the relative humidity, the greater was the number of fruits that shriveled. At a temperature of 4.5° C. and a relative humidity of 74 per cent, the number of fruits that shriveled by the end of 18, 25, and 32 days was greater in all four lots of experiment 2 (Table 7) than at a temperature of 10° and a relative humidity of 84 per cent—although the reverse was true after 11 days—as well as in experiment 1 (data not presented). A humidity of 74 per cent at 4.5° had a slightly greater saturation deficit than a humidity of 84 per cent at 10°, but this fact does not eliminate the difficulty, for there would still be a discrepancy because the effect of temperature is more than offset by this small difference in humidity. Of course, there is some room for error in the method employed in measuring shriveling. On the whole, the results show a marked increase in shriveling with the increase in temperature.

TABLE 7.—*Influence of temperature, humidity, and time on shriveling of green pepper fruits in storage*

Temperature	Relative humidity	Number of fruits used	Number of fruits shriveled after—					
			11 days	18 days	25 days	32 days	39 days	46 days
° C.	Per cent							
13	76	43	15	28	36	43		
13	71	43	31	42	42	43		
10	98	43	1	1	2	8	12	15
10	89	43	5	19	25	32	39	41
10	84	43	19	25	30	38	43	
4.5	95	43	0	1	1	5	9	10
4.5	87	43	6	8	18	18	35	39
4.5	74	43	15	32	42	43		
0	90	42	0	0	0	0	0	5
0	80	43	0	0	6	6	19	30
0	69	43	0	16	27	42	42	

The conditions most favorable for the storage of peppers from the standpoint of shriveling are (1) a temperature of 4.5° C. and a relative humidity of 95 per cent and (2) a temperature of 0° and a relative humidity of 90 per cent. The latter condition was somewhat more favorable than the former.

Taking into consideration the effects of temperature and humidity on infection and development of Botrytis rot and anthracnose and on ripening and shriveling, an examination of the results recorded in Tables 3 to 7 shows that the most favorable condition among those employed for the storage of peppers is a temperature of 0° C. and a relative humidity of 90 per cent. A temperature of 0° was the least favorable of those employed for the development of disease and for

shriveling and ripening. Although relative humidities of 69 and 80 per cent at 0° are less favorable for infection by *Botrytis* than a relative humidity of 90 per cent, those humidities brought about shriveling too rapidly for the prolonged storage of peppers. The limiting factor at a relative humidity of 90 per cent at 0° is infection by *Botrytis*, but the earliest infection was observed only after 32 days and the percentage of infection was not large until after 46 days.

SUMMARY AND CONCLUSIONS

Freezing injury and *Botrytis* rot of peppers are described. The lesions on pepper fruits caused by freezing are shown to be distinct from those produced by *B. cinerea*. The following distinguishing features may be emphasized: The lesions due to freezing injury have a water-soaked appearance, are a darker green than the normal fruit, and are indefinite in outline. The *Botrytis* lesions, on the other hand, are very definite in outline and are a creamy, buffy olive color.

It has been shown not only that *Botrytis cinerea* is able to infect pepper fruits when used in artificial inoculations, but that it does so normally and commonly at temperatures from 0° to 13° C.

Although peppers stored at temperatures fluctuating from 0° to 2° C. for a few weeks commonly become infected with *Botrytis cinerea*, occasionally there are lots that show little or no infection.

The number of pepper fruits infected by *Botrytis cinerea* out of the lots containing 129, 129, and 128 fruits, stored at temperatures of 0°, 4.5°, and 10° C., respectively, for 39 days, was 6 at 0°, 30 at 4.5°, and 10 at 10°. The temperature of 4.5° was more favorable for infection than either 0° or 10°, notwithstanding the optimum temperature for the growth of *B. cinerea* is about 25°. The time required for the initial infection to occur at 0° was about 32 days, as compared with 18 days at 4.5° and 10°.

There was an increase in the number of *Botrytis* infections with the increase in the relative humidity at each of the temperatures 0°, 4.5°, and 10° C.

Anthrachnose developed on pepper fruits contaminated with anthrachnose spores at all the temperatures employed (0° to 13° C.). The time required for infection to occur tended to increase as the temperature fell below 13°.

There was very little difference in the number of anthrachnose infections that occurred by the end of the various periods of time employed at temperatures of 4.5° and 10° C. The number of infections found at either of these two temperatures was much larger than at 0°. In general, the number of infections that occurred at 13° was larger than at the lower temperatures, notwithstanding that the two humidities employed at 13° were lower, on the basis of the saturation deficit, than at the lower temperatures. No consistent relation between relative humidity and infection with anthrachnose was found at the various temperatures.

Some ripening occurred at temperatures of 0°, 4.5°, 10°, and 13° C. In general, the number of fruits ripening decreased and the time required for ripening increased with the fall of the temperature below 13°. Very little ripening occurred in 39 days in fruits stored at 0° and 4.5°. Humidity did not seem to affect ripening in any way.

In general, the time required for shriveling to occur increased with the fall in temperature below 13° C. At all the temperatures employed, the number of fruits that shriveled after any given period of time increased rapidly with the lowering of the relative humidity. The time required for shriveling to occur decreased with the lowering of the humidity.

A temperature of 0° C. and a relative humidity of 90 per cent were more favorable for the storage of peppers than any of the other temperatures and humidities employed. This temperature is sufficiently above the freezing point of peppers (−1.06°) to insure against injury from freezing.

EXPERIMENTAL STUDIES ON THE GROWTH AND DEVELOPMENT OF STRAWBERRY PLANTS¹

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INTRODUCTION

The work reported in this paper constitutes a field study of the behavior of several varieties of strawberry plants during the greater part of the growing seasons of 1925 and 1926 at Glenn Dale, Md. Its purpose was to determine the environmental conditions most effective in promoting and limiting plant growth and development under field conditions. The general plan of the studies included detailed measurements of the plants growing in the field, especially of their leaf enlargement, in order to obtain numerical indices of their growth activity throughout the season, together with corresponding records of some of the main influential climatic conditions. The plant data were then compared with the climatic data to find out what correlations existed for the periods studied. The strawberry seems to be particularly adapted to studies on plant activity, because it is usually active from the opening of spring until the arrival of freezing weather, it is easily propagated vegetatively, and it is very tolerant of a wide range of climatic and soil conditions, although it responds with characteristic definiteness and promptness to slight climatic fluctuations. The results of the study show what conditions were most influential in the growth of the strawberry and present a general picture of its development throughout the growing season under Maryland conditions.

CLIMATIC RECORDS

The field records were taken at the United States Plant Field Station at Bell (post office Glenn Dale), Md. The work was begun in 1923 and continued through 1924, 1925, and 1926. As the principal records discussed herein are those of 1925 and 1926, climatic data for these years only are presented. Strawberries are commonly planted at the Bell station about April 1, and top growth usually ceases between October 15 and November 1. The growing season for tops therefore covers 6½ to 7 months, or about 210 days.

Air-and-soil thermograph records, records of white and black spherical porous porcelain atmometers, and records of soil-moisture content were taken in the field near the plants. Additional records were obtained from an official observation station of the United States Weather Bureau located but a few hundred feet from the strawberry field, and sunshine records were obtained from the weather station in Washington, D. C., about 15 miles distant.

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² Thanks are due Prof. B. E. Livingston, director of the laboratory of plant physiology of the Johns Hopkins University, for assistance in planning and conducting the experiments.

The atmometers were placed in the plant rows with the spheres from 6 to 10 inches above the ground and somewhat above the foliage. Readings were taken daily or at longer intervals at about 7.30 a. m.

Records of the soil-moisture content were obtained from April to July, 1925, for the most part weekly.³ Cores were taken in duplicate in each of two spots about 100 feet apart in the plant rows. Each core was divided into three parts to represent three different ranges in depth, 1 to 6 inches, 6 to 12 inches, and 12 to 24 inches. The upper 6 inches had the lowest and the second foot the highest average moisture content for the period for which the records were taken. A marked decrease in moisture content occurred early in June at the period of high temperatures, high evaporation, and high transpiration rates. At no other time was the moisture supply very low, however, and it is not probable that the soil moisture supply at the Bell station was often a limiting factor in the early development of these plants. The moisture content for the upper 6 inches was used for these records.

The sunshine records for the two years correspond closely with the radiation indices, but show minor differences.

PLANT RECORDS

In 1925 nine varieties—Howard 17, Klondike, Missionary, Dunlap, Parsons, New York, Aroma, Sample, and Gandy—were used in the experiments. These include the leading commercially grown varieties, which constitute over 80 per cent of the total strawberry acreage in the United States. Two varieties, Howard 17 and Klondike, were more thoroughly studied than the others, being taken as representative of the northern and of the southern sorts, respectively. In 1926 these two varieties were the only ones used in the studies.

In 1925, 100 plants of each variety were selected for uniformity in size and general appearance and according to the dates when they rooted in 1924. On April 1 they were set in adjoining rows, and after becoming established the 10 plants of each variety at one end of each row were chosen for special study as representative of the 100 of each sort. Conditions were very favorable for the growth of these plants in 1925, and all made satisfactory development.

In the spring of 1926, 100-plant rows of Howard 17 and Klondike were set and a group of 12 plants near the north end of each row was selected for study. Growth was satisfactory but not as rapid in the early part of the season as in 1925.

The plan to obtain plant records at intervals of one week throughout each of the two seasons was generally followed, although some of the intervals were modified in both seasons. At each observation each new leaf that had expanded until most of its upper surface was exposed was numbered and tagged. Likewise, each new runner was numbered and tagged, or removed if that was required by the plan. All leaves and runners were then measured and recorded. The plant records, therefore, showed the complete history of each leaf and runner, including the leaves that developed at the tip of each runner. As runner tips became ready to take root they were pegged down in

³ The soil-moisture determinations were made by J. W. McLane, assistant biophysicist, Biophysical Laboratory, Bureau of Plant Industry.

position for rooting. In both 1925 and 1926 some of the selected plants were used for a study of their behavior after runners were removed. For this purpose, beginning June 17, 18, or 19 for different plants and continuing to the end of the growing season of 1925, all runners were removed from 5 of the 10 plants of each variety. In 1926 the runners were removed from 10 of the 12 plants, only 2 being allowed to form clons.

The records included leaf measurements, runner measurements, and records of the position of runners. The length and breadth of each of the three leaflets of each leaf and the length of each runner were obtained with a flexible centimeter rule. The length and the breadth were multiplied together to give the leaf product for each leaflet, and the products for the three leaflets were summed to give the leaf product for each leaf. The sum of the leaf product of all leaves gave the leaf product in square centimeters for the entire plant, which is used as an index (I) of its leaf area. To obtain the leaf-product increment for the interval the leaf product at the beginning of an interval was subtracted from that obtained at the end. The mean daily increment in leaf product for an interval was obtained by dividing the leaf-product increment for the interval by the number of days in the interval. Finally, the mean daily percentage increment in leaf product for a period was calculated in two ways, as follows: (1) By dividing the mean daily increment for the interval by the leaf product obtained at the beginning of the interval and multiplying by 100 to give index I_1 ; (2) by dividing the mean daily increment for the interval by the mean leaf product for the interval and multiplying by 100 to give index I_2 . Although the plants grew vigorously, as shown by their indices of leaf area (I) their mean daily increment and the indices I_1 and I_2 are relatively small.

In these studies the mean daily percentage increment in leaf product for any interval (I_2) is taken as an index of plant activity for that interval. The value of this index changes throughout the season, and its fluctuations are very nearly the same as the fluctuations in the rate of production of leaf surface and in the capacity of the plant to produce food. Leaf production is one of the main forms of activity in strawberry plants and may be considered as an index of general activity or growth.

Some special tests were made to determine the relation between these leaf-product values and actual leaf areas. Actual leaf area (by planimeter measurements) and leaf products were obtained for the same leaves of the Howard 17 variety. The results showed that actual leaf area was about 75 per cent of the corresponding leaf-product value with very slight variation. The leaf-product value for the entire leaf was much more satisfactory than was that for the terminal or for either of the lateral leaflets, and the leaf-product value for the terminal was more satisfactory than that of either lateral leaflet or that of the two laterals taken together. It was also found that the leaf product used in the tests was very much more satisfactory than any linear dimension, such as length of the leaflets.

The three indices (I , I_1 , and I_2) for each variety and interval from April 1 until June 9 are set forth in Table 1.

TABLE 1.—Leaf-product indices (I , I_1 , and I_2) for nine strawberry varieties, 10 plants of each, by intervals from beginning of the growing season until about the beginning of runner formation, 1925

Variety	Kind of index	Indices for interval No —							
		0	1	2	3	4	5	6	7, 8
		Apr 1-14	Apr. 15-21	Apr. 22-28	Apr. 29-May 5	May 6-12	May 13-20	May 21-26	May 27-June 9
Howard 17..	I	53	91	134	168	235	397	578	1,091
	I_1		10.2	6.7	4.6	5.7	8.7	7.6	6.3
	I_2		7.5	5.4	4.0	4.7	6.5	6.4	4.4
Missionary	I	21	44	81	105	166	278		^a 874
	I_1		15.7	12.0	4.2	8.3	9.0		^a 10.2
	I_2		10.0	8.4	3.7	6.4	6.3		^a 5.2
Dunlap.....	I	18	33	54	68	96	160		501
	I_1		11.7	9.1	3.7	5.9	^b 9.5		^c 9.7
	I_2		8.1	6.8	3.3	4.9	^b 7.1		^c 4.7
New York.....	I	27	45	73	97	159	258		^c 800
	I_1		9.6	8.9	4.7	9.2	8.9		^c 9.5
	I_2		7.1	6.8	4.0	6.9	7.1		^c 4.7
Aroma.....	I	19	34	57	72	115	162		^d 415
	I_1		11.1	9.7	3.7	8.5	5.8		^d 6.8
	I_2		8.1	7.1	3.3	6.5	4.9		^d 3.8
Gandy	I	19	35	59	79	106	187	^e 446	589
	I_1		12.1	9.7	4.9	4.9	10.9	^e 9.7	3.6
	I_2		8.5	7.3	4.2	4.2	7.9	^e 5.8	3.1
Average of 6 varieties	I_1		11.7	9.4					
	I_2		8.2	7.0					
Klondike ..	I	37	60	107	144	219	342	493	986
	I_1		8.9	11.2	4.9	7.4	7.0	7.4	7.1
	I_2		6.7	8.0	4.2	5.9	5.4	6.0	4.8
Parsons ..	I	24	34	59	81	136	227		^c 728
	I_1		5.8	10.6	5.3	9.8	9.6		^c 10.0
	I_2		4.9	7.6	4.5	7.2	7.1		^c 4.8
Sample.....	I	28	50	96	118	172	243	^e 468	749
	I_1		11.1	13.2	3.2	6.5	5.9	^e 6.5	^f 6.7
	I_2		8.1	9.0	2.9	5.3	4.9	^e 4.5	^f 5.1
Average of 3 varieties	I_1		8.6	11.7					
	I_2		6.6	8.2					
Average of 9 varieties	I_1				4.4	7.4	8.4	8.5	8.3
	I_2				3.8	5.8	6.2	5.7	4.9

^a For interval May 21 to June 9.

^b For interval May 13 to 19.

^c For interval May 20 to June 10

^d For interval May 20 to June 11.

^e For interval May 20 to June 2

^f For interval June 3 to June 11.

At the end of the last interval shown in Table 1 (ending June 9, 1925), the 10 plants of each variety were divided into two groups of five plants each and all runners were removed from the plants of one group as they were produced. Measurements were discontinued at different times for the different varieties as shown in Table 2.

TABLE 2.—Leaf-product indices (I , I_1 , and I_2) for nine strawberry varieties, five plants of each with runners left on and five plants of each with runners removed, by intervals from about the beginning of runner formation until the end of observations, 1925

Variety and group	Kind of index	Indices for interval No.—									
		9	10	11	12	13	14	15	16	17	18
		June 10-16	June 17-23	June 24-30	July 1-7	July 8-14	July 15-22	July 23-28	July 29-Aug. 6	Aug. 7-19	Aug. 20-Sept. 16
<i>Howard 17</i>											
With runners -----	<i>I</i>	*1,346	1,772	2,323	2,885	3,541	4,035	4,781	7,043	10,669	*16,551
	<i>I</i> ₁	*3 2	4 8	4 4	3 5	3 2	1 8	3 1	5 3	4 0	*2.1
	<i>I</i> ₂	*2 2	4 1	3 8	3 1	2 9	1 7	2 8	4 3	3 1	*1.6
Without runners -----	<i>I</i>		1,388	1,623	1,879	2,138	2,184	2,375	2,704	2,886	*4,590
	<i>I</i> ₁		1.6	2 6	2 3	2 0	.3	1.4	1 7	0.5	*0.7
	<i>I</i> ₂		1.5	2 2	2 1	1 8	3	1 3	1 4	.5	*.7
<i>Klondike</i>											
With runners..	<i>I</i>	1,389	1,772	2,409	2,963	3,682	4,566	45,890		*16,762	*2,427
	<i>I</i> ₁	3.5	4.0	5 1	3 3	3 5	*2.6	*4 8		*6 8	/ 2.1
	<i>I</i> ₂	3 1	3 5	4 4	2 9	3 1	*2.4	*4 2		*3 6	/ 1.2
Without runners -----	<i>I</i>	959	1,130	1,362	1,606	1,786	2,097	*2,244		*2,809	*1,775
	<i>I</i> ₁	1 7	2 5	2 9	2 6	1 6	*1 9	*1 2		*1 0	*-0.5
	<i>I</i> ₂	1 6	2 3	2 7	2 3	1 5	*1 8	*1 0		*.8	*- .6
<i>Missionary</i>											
With runners..	<i>I</i>	1,095	1,434	1,909	*3,253						
	<i>I</i> ₁	4 7	4 1	4 7	*4 4						
	<i>I</i> ₂	4 1	3 8	4 1	*3 3						
Without runners -----	<i>I</i>	209	966	1,221	*1,682						
	<i>I</i> ₁	2.3	2 8	3 8	*2 4						
	<i>I</i> ₂	2 1	2 5	3 3	*2 0						
<i>Dunlap</i>											
With runners.....	<i>I</i>	*511	*777	1,010	*1,916						
	<i>I</i> ₁	*3 7	*4 5	4 3	*5 6						
	<i>I</i> ₂	*3 3	*4 0	3 7	*3 9						
Without runners -----	<i>I</i>	*574	*670	827	*1,194						
	<i>I</i> ₁	*2 9	*2 8	3 4	*2 8						
	<i>I</i> ₂	*2 7	*2 6	4 0	*2 5						
<i>Parsons</i>											
With runners.....	<i>I</i>	*842	*1,087	1,528							
	<i>I</i> ₁	*5 2	*4 8	5 8							
	<i>I</i> ₂	*4.4	*4 2	4 8							
Without runners --	<i>I</i>	*853	*1,068	*1,194							
	<i>I</i> ₁	*1.7	*4.2	3 2							
	<i>I</i> ₂	*1 6	*3.7	2 9							
<i>New York</i>											
With runners -----	<i>I</i>	*917	*1,107	*1,438		*2,567					
	<i>I</i> ₁	*4 2	*4 1	*3 7		*3 2					
	<i>I</i> ₂	*3.6	*3.7	*3.3		*2.4					
Without runners -----	<i>I</i>	*1,075	*1,293	*1,452		*2,823					
	<i>I</i> ₁	*2.7	*4 1	*1.5		*2.0					
	<i>I</i> ₂	*2 4	*3 7	*1.4		*1.2					
<i>Aroma</i>											
With runners.....	<i>I</i>	*518									
	<i>I</i> ₁	*4.1									
	<i>I</i> ₂	*3.6									
Without runners -----	<i>I</i>	*441									
	<i>I</i> ₁	*4.4									
	<i>I</i> ₂	*3.8									

* Indices for all 10 plants

^b Average of 2 plants instead of 5 plants.

* For interval July 15 to 23.

* For interval July 24 to 30.

* For interval July 31 to Aug. 20.

/ For interval Aug. 27 to Sept. 18 and average of 2 plants instead of 5 plants.

* For interval July 1 to 16.

* For interval June 11 to 17.

* For interval June 18 to 23.

* For interval June 11 to 18.

* For interval June 19 to 23.

* For interval June 24 to July 1.

* For interval July 2 to 23.

* For interval June 12 to 18.

TABLE 2.—*Leaf-product indices (I , I_1 , and I_2) for nine strawberry varieties, five plants of each with runners left on and five plants of each with runners removed, by intervals from about the beginning of runner formation until the end of observations, 1925—Continued*

Variety and group	Kind of index	Indices for interval No.—									
		9	10	11	12	13	14	15	16	17	18
		June 10-16	June 17-23	June 24-30	July 1-7	July 8-14	July 15-22	July 23-28	July 29-Aug. 6	Aug. 7-19	Aug. 20-Sept. 16
<i>Sample</i>											
With runners	I	*982									
	I_1	*4.6									
	I_2	*4.0									
Without runners	I	*762									
	I_1	*2.1									
	I_2	*1.9									
<i>Gandy</i>											
With runners	I	*741									
	I_1	*3.3									
	I_2	*2.9									
Without runners	I	*787									
	I_1	*3.8									
	I_2	*3.4									
<i>Average of all varieties</i>											
With runners	I_1	4.4	4.4	4.7	3.4	3.4	2.2	4.0	5.4	5.3	2.5
	I_2	3.7	3.6	4.0	3.0	3.0	2.1	3.5	4.7	3.3	1.6
Without runners	I_1	3.1	2.9	2.9	2.5	1.8	1.2	1.3	1.4	.8	2
	I_2	2.6	2.7	2.6	2.0	1.6	1.1	1.2	1.2	.7	1

* For interval June 12 to 18.

In 1926 the period of observations extended from June 26 to October 9. The index values obtained are shown in Table 3. Two series of values are shown for each variety, one for the 2 plants with runners (these becoming clons) and the other for the 10 plants without runners.

TABLE 3.—*Leaf-product indices (I , I_1 , and I_2) for two strawberry varieties, 2 plants of each with runners left and 10 of each with runners removed, by intervals from about the beginning of the season of runner formation until about the end of the growing season, 1926*

Variety and group	Kind of index	Indices for interval No —											
		1	2	3	4	5	6	7	8	9	10	11	12
		June 26- July 6	July 7-12	July 13-19	July 20-26	July 27- Aug. 2	Aug. 3-9	Aug. 10- 16	Aug. 17- 23	Aug. 24- 30	Aug. 31- Sept. 7	Sept. 8-21	Sept. 22 to Oct. 7-9
<i>Howard 17</i>													
With runners.....	<i>I</i>	871	1,269	1,526	1,905	2,610	2,899	2,618	3,419	3,934	5,099	8,433	12,954
	<i>I</i> ₁	4.7	6.5	2.9	3.5	5.3	1.6	-0.6	3.2	2.2	5.3	4.0	3.3
	<i>I</i> ₂	3.7	5.3	2.6	3.2	4.5	1.5	-1.6	2.7	2.0	4.6	3.1	2.6
Without runners.....	<i>I</i>	738	1,014	1,108	1,307	1,590	1,673	1,348	1,581	1,586	1,897	2,148	2,655
	<i>I</i> ₁	4.3	8.9	1.3	2.4	2.7	1.0	-2.8	2.6	0	2.3	0.9	1.5
	<i>I</i> ₂	3.5	7.8	1.3	2.2	2.5	1.0	-3.1	2.3	0	2.1	.9	1.3
<i>Klondike</i>													
With runners.....	<i>I</i>	812	849	1,168	1,287	1,604	1,604		2,124	2,518	3,308	4,452	5,614
	<i>I</i> ₁	3.9	0.7	5.4	1.5	4.2	-0.5		2.3	2.6	3.9	2.5	1.4
	<i>I</i> ₂	3.2	0	4.2	1.4	3.7	-1.5		2.0	2.4	3.4	2.1	1.2
Without runners.....	<i>I</i>	410	456	635	758	859	845		788	565	864	940	1,022
	<i>I</i> ₁	5.3	1.9	5.6	2.8	1.9	-0.2		-0.5	-4.0	6.6	0.6	0.5
	<i>I</i> ₂	4.1	1.8	4.7	2.5	1.8	-2		-0.5	-4.7	5.2	.6	
<i>Average</i>													
With runners.....	<i>I</i> ₁	4.3	3.6	4.2	2.5	4.8	.6		2.8	2.4	4.6	3.3	2.4
	<i>I</i> ₂	3.5	3.0	3.4	2.3	4.1	.5		2.4	2.2	4.0	2.6	1.9
Without runners.....	<i>I</i> ₁	4.8	5.4	3.5	2.6	2.3	.4		.7	-2.0	4.5	.8	1.0
	<i>I</i> ₂	3.8	4.8	3.0	2.4	2.2	.4		.6	-2.4	3.7	.8	.9

* For interval Aug. 10 to 23.

When the intervals in Tables 1, 2, and 3 were the same for all varieties considered, the average index values were derived in the ordinary manner; when the intervals differed so that the averages could not be obtained in the ordinary way, those for the actual intervals used were first plotted as ordinates on a graph with these intervals as abscissas, and the average values were read from the graph for the dates required.

Because the varieties Klondike, Parsons, and Sample behaved differently from the remaining six for the first two intervals of 1925 (Table 1), the averages for these intervals were determined for these three varieties and for the remaining six separately. The two groups behaved similarly throughout the summer after the first two intervals, and for later intervals their index values were combined in single averages and taken to represent both groups together.

The data for climatic values and for leaf-product indices are presented in Figures 1 and 2. Figure 3 shows the appearance of a part of the experimental rows in 1926 with leaves tagged and runner plants pegged.

DISCUSSION

COMPARISON OF LEAF-PRODUCT AND CLIMATIC GRAPHS

A comparison of the plant graphs in the lower parts of Figures 1 and 2 with the climatic graphs in the upper parts of the same figures brings out the fact that the two sets of graphs are not generally in agreement for either season. The seasonal march of the leaf-product indices does not follow the march of any one of the climatic indices here used, but shows the influence of different climatic factors. The first two intervals of 1925 may be disregarded as being especially influenced by genetic differences. The second index (I_2) appears more useful than the first (I_1) and is used in the discussion.

The first elevation of the leaf-product graph for 1925 (intervals 4 to 7) corresponds to temperature, evaporation, radiation, and sunshine-duration values from low to medium, and to high soil-moisture values. The second elevation (interval 16) for the clons, corresponds to a definite depression in the temperature graph and to low values of evaporation, radiation, sunshine duration, and high soil-moisture content. The first elevation of the leaf-product graph for 1926 (interval 5) corresponds to a depression in the temperature-maximum graph, to an elevation in the temperature-minimum graph, and to relative depressions in the graphs for evaporation, radiation, and sunshine duration. The second elevation (interval 10) corresponds to medium values for temperature, evaporation, and radiation, and to a low value for sunshine duration.

The first depression of the graph for 1925 (interval 3) corresponds in time to exceptionally low values for all four temperature indices and to relatively low values for evaporation, radiation, and sunshine duration, but the soil-moisture content for this interval is high. The broad second depression for 1925 (intervals 9 to 15) corresponds to high temperature values throughout, to low or medium soil-moisture values, to high evaporation and radiation values, and to medium sunshine-duration values.

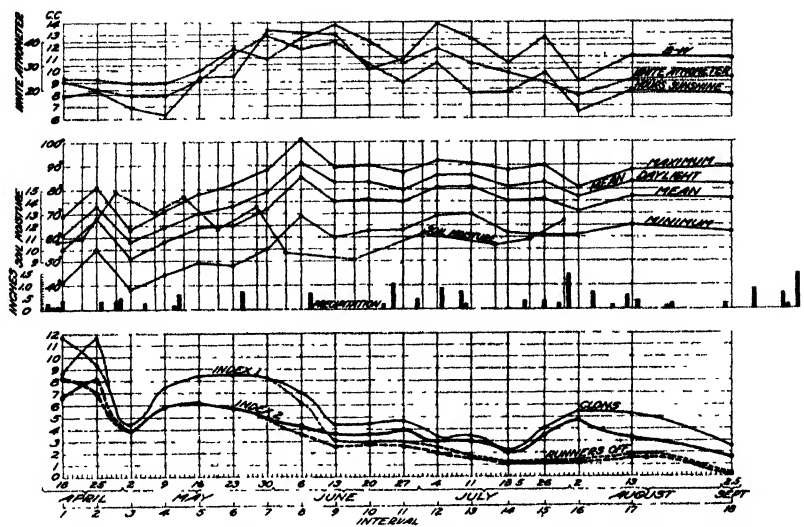


FIGURE 1.—Plant activity shown in terms of leaf-product indices (I_1 and I_2) of the strawberry for 1925 at the United States Plant Field Station, Bell, Md., together with temperature, rainfall, soil-moisture, evaporation, and sunshine graphs for the same period

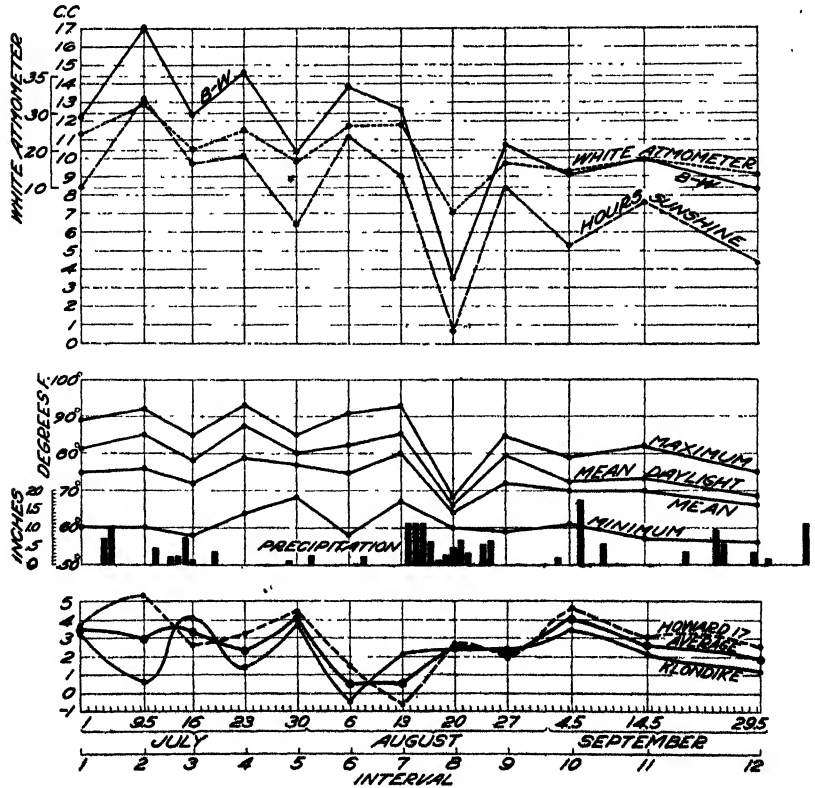


FIGURE 2.—Plant activity shown in terms of leaf-product indices (I_2) in Klondike and Howard 17 strawberry plants and of their average for 1926 with corresponding climatic data



FIGURE 3.—Strawberry plants, Howard 17 (at left) and Klondike (at right), on which growth studies were made in 1926. The plant of Howard 17 in the immediate foreground had all runners removed as fast as formed, while the next plant had its runners pegged to the soil in order to induce them to root promptly

The pronounced depression of the leaf-product graphs for 1926 does not correspond to any of the climatic graphs thus far considered, but it is to be remembered that there is no soil-moisture graph for this season. However, an examination of the precipitation graph shows that this depression of the leaf-product graph represents, in time, the end of a long drought period, and it is safe to suppose that the soil-moisture values at that time were very low.

Considering now only the plant graphs and those for average mean daylight temperature, some indications as to the air-temperature conditions that apparently have been especially favorable or unfavorable for rapid leaf production may be reached. In both seasons high leaf-product values occur for intervals with daylight-temperature values approximately between 64° and 80° F., and low leaf-product values occur for intervals with daylight-temperature values of about 79° or above, and also of 68° or below. These observations, together with many details shown on the graphs, lead to the conclusion that temperature was generally the limiting factor in the production of leaves on the plants used in these studies. In these two seasons the moisture conditions were probably generally adequate, with the exception noted for intervals 6 and 7 of 1926, and no specific influence can be postulated for the conditions of evaporation, radiation, and sunshine duration except in those cases where temperature influence was also indicated. It may therefore be supposed that there was a range of daylight-temperature values from about 64° to 80° within which leaf production went on at high rates, whereas daylight-

temperature values higher or lower than this range gave characteristically low rates of leaf production. It is to be noted that these limits are not exactly the same when derived from the occurrence of high leaf-product values as when derived from the occurrence of low leaf-product values. From these results it might be concluded that the optimum value for the daylight mean is not far from 73° (perhaps between 70° and 76°). This same optimum has been derived by studying these graphs in several other ways, and it may be taken tentatively as approximately correct for the data at hand.

Though plant activity as measured by the leaf-product method indicates low rates below 64° and above 80° F., it should be emphasized that this applies to the plants under the conditions of this study. The responses might be different with the long days in Alaska and the varieties grown there or under irrigation at high elevations in Western States. However, because the varieties included the leading sorts grown in the United States and because of the variety of climatic conditions encountered, it is probable that the activity of tops during most of the growing period responds in a similar manner to these temperatures in the humid regions of the United States. Strawberries are raised successfully in the South, where high temperatures are often encountered during the summer, because there some growth can take place from time to time throughout the long growing season and because of the occurrence of periods with temperatures near the optimum in fall and spring. In the North the strawberry succeeds because the temperatures are near the optimum during much of the summer, even though they are below the optimum during most of the spring and fall.

SEASONAL MARCH OF MATURE-LEAF SIZE AS INDICATED BY MEAN LEAF PRODUCT

The measurement at regular intervals of each leaf on all plants in these studies made it possible to obtain data showing how the leaf size of mature leaves was related to the time of year when they unfolded from the bud, and to their position, whether on parent or runner plants; also, how the production of runner plants influenced mature-leaf size in the mother plants. Four plants of the Howard 17 variety were used for this study. Two were of the group allowed to produce runners and to form clons, and the other two were of the group from which all runners were removed. The leaves were classed according to the dates on which they were first observed unfolding from the bud. After the beginning of runner-plant formation the data for the two plants with runners removed were separated from those for the two producing runner plants. In addition, the runner plants themselves were classed according to the dates on which they were first observed as plants. The data obtained from these leaf measurements were plotted to form the graphs shown in Figure 4.

It will be noted that the graph for mother plants rises at first slowly then rapidly until June 10, when runner plants began to be formed. (Fig. 4.) After June 24, the graph for mother plants with runners (producing runner plants) shows a decided drop, which continues, except for a slight rise July 8, until the end of the period. The graph for mother plants with runners removed maintains its high level, rising from July 1 to July 23. For the period from the

beginning of observation to the beginning of runner-plant formation the leaves of successive leaf classes were progressively larger when enlargement had ceased, that is, the later they unfolded from the bud the larger they became. When runners were removed as they formed, thus preventing the formation of runner plants and the presumable corresponding drain on the mother plant, the leaves of later leaf classes became even larger. But when runner plants were allowed to form, the period of runner-plant formation was characterized by smaller mature leaves on the mother plant. The greatest mean leaf product is for the leaf class of August 7, and its value is 160, which is more than four times as great as the corresponding mean for leaves of the first leaf class (April 15) which had passed the winter in the buds. For the mother plants that were

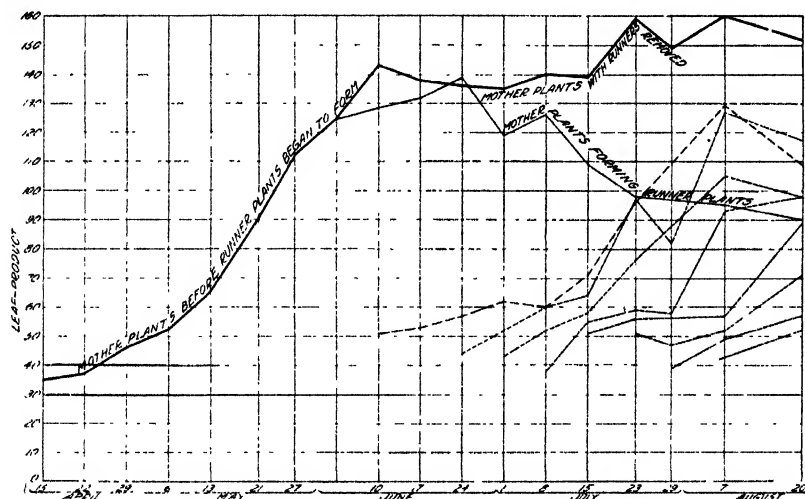


FIGURE 4.—Graph showing (for plants of Howard 17) average size of mature leaves (leaf product) for each leaf class. For mother plants before runner plants began to be formed (broad line before June 10); for mother plants with runners removed (broad line after June 10); and for mother plants forming runner plants (narrow line), as well as for runner plants of each runner-plant class (broken lines). Abscissas represent leaf classes (designated by date on which leaves unfolded from bud) and runner-plant classes (designated by date on which runner plants were formed), ordinates are the average leaf products

allowed to produce runner plants the leaves of the leaf class of August 7 gave a mean leaf product of only 95 when enlargement ceased. Thus, the removal of runners had increased the mean leaf product for this leaf class by more than 50 per cent.

Turning to the graphs (fig. 4) for mean leaf products of leaves on runner plants, it may be noted that the first leaf class in each runner-plant class gave a mean leaf product of the same general order of magnitude as that given by leaves of the first three or four leaf classes of the mother plants, and this seems to be true without reference to the runner-plant class. That is, the first leaves produced by a runner plant apparently attained about the same size when mature, whether the plant took root June 10 or August 7 or at any intervening time, and the size attained was practically the same as that reached by mother-plant leaves that unfolded at the beginning of the season.

This relation is especially striking when it is recalled that the environmental conditions of early spring differed greatly from those of mid-summer.

Furthermore, the graphs for successive runner-plant classes closely resemble in shape the graph for the parent plants before runner plants began to form. For any runner-plant class the graph slopes upward, at first slowly and then more rapidly. In the first three runner-plant classes (June 10 and 24 and July 1) a maximum in mature-leaf size is shown for the leaves of the leaf class of August 7, just as in the case of the leaves of the mother plants from which runners were removed. The actual maximum leaf products for the first two runner-plant classes (June 10 and 24) are much smaller (129 and 127) than the corresponding value for the plants with runners removed (160), but much greater than that for the mother plants producing runner plants (95). The maximum for the third runner-plant class (July 1) is smaller, but still greater than the mother-plant value just mentioned. The succeeding five runner-plant classes each gave progressively larger mature leaves with successive leaf classes, but apparently the growing season was not long enough to allow the graphs of these runner-plant classes to show maxima. At any rate, for these last five runner-plant classes, leaves of the leaf class of August 20 gave higher mean leaf products at maturity than did those of the leaf class of August 7.

It seems to be indicated that under the conditions of this test, with apparently ample moisture, the size of a runner-plant leaf at maturity was determined largely by the age of the runner plant at the time the leaf unfolded, rather than by the special environmental conditions prevailing either at the time the runner plant rooted, at the time the leaf unfolded, or during the period of enlargement of the leaf.

From the results of this study of mature-leaf size the following picture of some aspects of the development of the strawberry plants dealt with may be tentatively presented. The early developmental stages of the spring-set plant consist in a rapid enlargement of the leaf and root systems by which the rate of net income in all necessary materials is rapidly increased; enlargement of leaf and root systems and the general maintenance of health cost the plant a considerable quantity of essential materials, but the increased income more than offsets these costs from a very early period of development. A larger root system results in a more rapid supply of water and salts from the soil, and a larger leaf system results in a more rapid production of the products of photosynthesis. Leaves that unfold later are therefore better supplied with needed materials than are those that unfold earlier. The later ones should therefore approach the genetic maximum leaf size for the variety considered. But the production of runners and runner plants soon begins, and these are more active in growth than the crown of the parent plant, apparently actually decreasing its supply of necessary materials and limiting the enlargement of leaves that unfold from the crown after runner formation has set in. For a time the runner plant seems to hold the advantage, and its leaves become larger than those contemporaneously developed from the crown, thus approaching the maximum leaf size for the given variety. In its early stages of development the runner plant receives from the parent crown relatively large quantities of water, salts, and the products of photosynthesis. It soon forms, in addition, large

quantities of the products in its own rapidly enlarging leaf system, and derives additional water and salts through its own root system, especially since its roots enter regions of soil not previously explored by the roots of the parent plant.

In turn, the runners and runner plants produced by the first runner plants to be formed from a mother plant may indirectly affect the mother plant itself to some extent, and their relations to the first runner plants are in general the same as those that obtain between the original crown and the first runner plants.

LONGEVITY OF LEAVES AS RELATED TO TIME OF UNFOLDING

Although the strawberry is an evergreen plant, at the Bell station its individual leaves do not live even throughout the growing season. The first leaves to die are those that passed the winter in the expanded mature condition and the next are those that were partially developed in the preceding fall and passed the winter in the bud. Leaves produced entirely in the spring live longer than either overwintering kind.

To study the relative length of life of leaves that unfold at different times in the growing season, data derived from the five plants of the Howard 17 variety with runners removed were employed. The leaves were serially numbered, the length of life was determined for each leaf, and the resulting values were grouped by leaf classes and by length of life in each class. These data include 12 leaf classes from April 15 to July 8, 1925, for all five plants grouped together. Each class includes all leaves first observed as unfolded on the date representing their class. In any leaf class there was considerable variability in leaf longevity, but all leaves of each of the first seven classes (April 15 to May 27) were observed to have died by August 6. Some leaves in each of the later classes were still alive on August 10 when observations on time of death were discontinued.

The results obtained are presented in Figure 5. Approximate dates of unfolding and of death are shown as abscissas, and the serial numbers of the 118 leaves that were studied are shown as ordinates. The height of the shaded blocks at the left-hand end indicates the serial numbers of the leaves and consequently the total number in each leaf class. Each block is bounded at the right by a stepped line the vertical portions of which show the approximate dates of death of the corresponding leaves. The lowest serial leaf numbers in each class refer to the shortest lived leaves of the class. For example, 10 leaves were observed as unfolded on April 15, and these are serially numbered from 1 to 10; therefore the leaf class of April 15 included leaves Nos. 1 to 10. Eight of these (Nos. 1 to 8) were recorded as dead on June 10 and the remaining two (Nos. 9 and 10) on June 17. The second leaf class included six leaves (Nos. 11 to 16). Of these, one died June 10, three June 17, one June 24, and the remaining one July 1. The blocks for later leaf classes may be interpreted in a similar manner by reference to the marginal scales. Of the 118 leaves studied, 87 had died by August 19. Their length of life ranged from 21 to 77 days and averaged 56 days. It may therefore be said that the mean or normal longevity of these summer leaves was nearly two months.

In a general way the leaves died in sequence, those of the earlier classes dying first; but exceptions will be noted, doubtless owing to peculiar conditions operating in these cases. It is not possible to state

just why some leaves of a given class died before others of the same class. Leaf-spot diseases (caused by infection by *Macrospirella* or *Dendrophoma*) may have been a contributing factor, but general observation led to the conclusion that infection was not generally important in determining the dates of death of an infected leaf. Infection was not usually observed until the leaves were mature or past maturity. Old leaves may have offered exceptionally favorable conditions for infection. The appearance of these leaf spots almost exclusively on older leaves can hardly be related to a more or less fixed period necessary for the preliminary development of the fungus within the tissues, for other varieties susceptible to these diseases showed severe injury within a few days after the leaves unfolded.

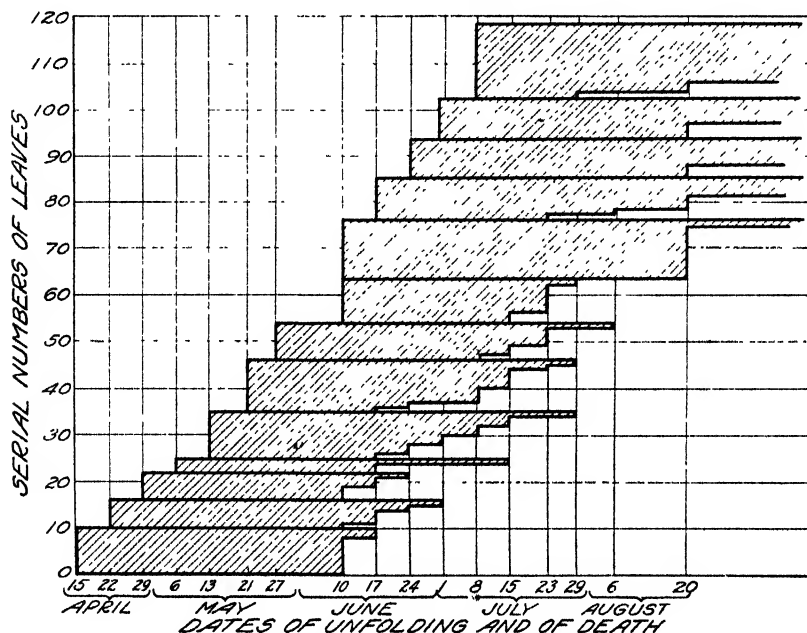


FIGURE 5 Length of life of all leaves produced by five plants of the Howard 17 strawberry with runners removed, from April 1 to July 8, 1925

COMPARISON OF VARIETIES WITH RESPECT TO NET LEAF PRODUCTION

Seven different varieties were compared with respect to the seasonal march of the value of the average total leaf product per plant, and the results are shown graphically in Figure 6, the data for which are derived from Tables 1 and 2. Ten plants of each variety, together with their runner plants, were used in obtaining the average values. The scale of values of the average total leaf product per plant is shown at the left, and the progress of the season (from April 15 to July 1, 1925) is shown by points and dates along the base line.

For all varieties development is seen to have been slow until about May 13, after which net leaf production was more rapid. In general the relative position of the several lines remains the same throughout their course; varieties with larger values at the beginning of this period had larger values throughout, for there is little crossing of the

lines of the figures. The most active variety was Howard 17 and the least active was Aroma. The latter generally makes but poor growth on the sandy soil of Bell station.

COMPARISON OF NET LEAF PRODUCTION IN PLANTS WITH RUNNERS REMOVED
AND WITH RUNNERS LEFT ON AND ALLOWED TO ROOT

The seasonal march of the average value of the total leaf product per plant was studied for the period from April 15 to August 20, 1925,

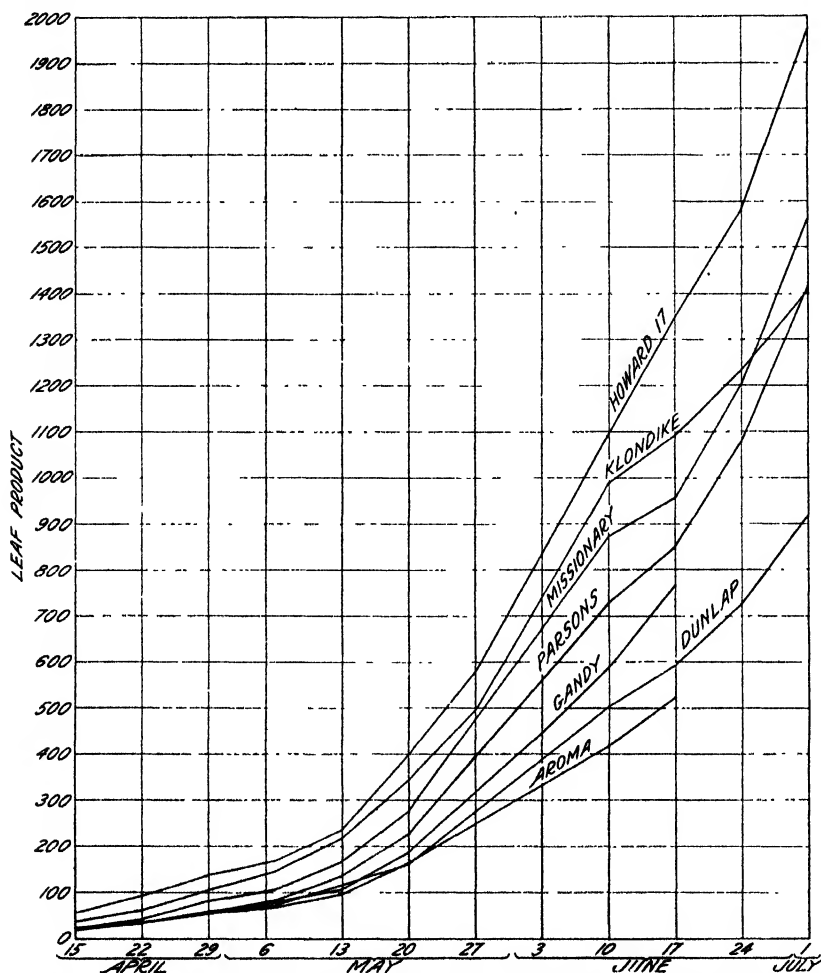


FIGURE 6.—Seasonal marches of total leaf area per plant (as indicated by average values of total leaf product per plant) for each of seven different varieties of strawberry. Ten plants with their runner plants were used for each variety, and all were set out April 1, 1925

for plants with runners removed and with runners left and allowed to produce runner plants. In the latter case the runner-plant leaves were not included. Five plants were included in each group. The average values (Tables 1 and 2) are shown graphically in Figure 7 by the continuous line marked "Runners off average," representing

those from which runners were removed as formed. In this figure are shown also narrow-line graphs for the minimum value of the leaf product and for the maximum value corresponding to the two main graphs. These minimum and maximum values are not shown in Tables 1 and 2, but have been calculated from the original observational data.

It is seen that the average rate was slow till about May 13, after which it was more rapid but nearly uniform to the end of the period in the case of the runnerless plants, but only until about July 1 for the plants that were allowed to produce runner plants. After about July 15 the average graph for the latter group of plants descends slightly and then remains nearly horizontal to August 20, when these observations were discontinued. It is thus again indicated that the vigor of the parent plant was greatly increased by the removal of the runners as they appeared. In other words, the production of runners and runner plants limits the growth vigor of the parent plant through-

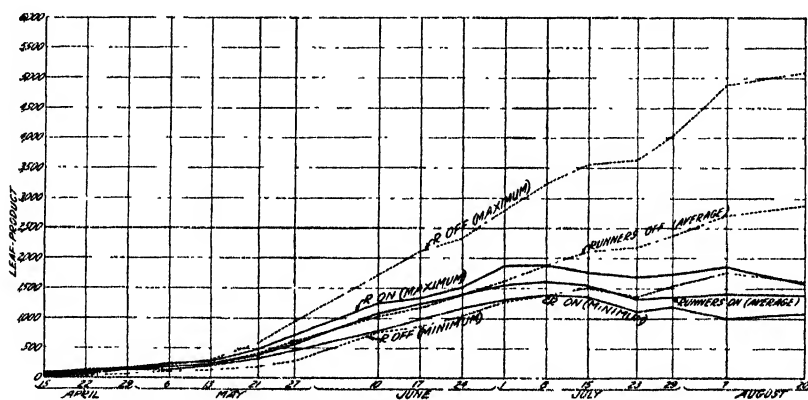


FIGURE 7.—Seasonal marches of total leaf area per plant (as indicated by values of total leaf product per plant) for plants of the Howard 17 variety of strawberry, with runners removed and with runners left and allowed to root. Five plants were included in each group, all were set out April 1, 1925

out the latter part of the season. On August 20 the average value of the total leaf product per plant of the plants without runners was nearly twice as great as that of the plants with runners.

It is to be noted, however, that the maximum deviation from the average is greater for the plants with runners removed than for the others, and that this difference becomes greater with the advance of the season.

COMPARISON OF NET MEAN DAILY INCREMENTS OF MEAN LEAF-PRODUCT VALUES FOR PLANTS WITH RUNNERS REMOVED AND WITH RUNNERS RETAINED

The seasonal fluctuations in the value of the mean daily increment of leaf production were determined in terms of the corresponding leaf-product increments for the period from April 1 to August 20, 1925. The data for these daily increments were derived from those given in Tables 1 and 2 for the plants of Howard 17. Ten plants were used before June 17, after which five plants with runners were used and five with runners removed. The results are presented in Figure 8.

For the first four intervals (April 1 to May 6) the value of the daily increment was about five, or nearly constant. For the next four intervals (May 6 to June 10) it increased until a maximum of 34 was reached for the last of these four intervals. From the interval beginning June 17 to that beginning July 15 the plants with runners removed showed steadily decreasing increment values until these values became negative (indicating net losses of leaf area) for the intervals beginning July 8 and July 15. These plants showed small positive values for the next two intervals and a small negative value for the interval beginning August 7.

The plants with runners gave positive increment values for all intervals, with much fluctuation. The values are exceptionally low for the intervals beginning July 15 and August 7.

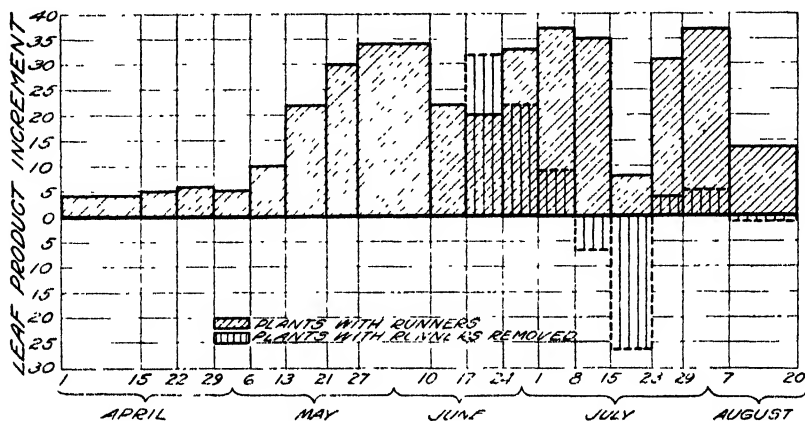


FIGURE 8.—Mean daily increments of the average value of the total leaf product per plant for each observational interval from April 1 to August 20, 1925. Before June 17, 10 plants were employed; for the rest of the period 5 plants without runners and 5 with runners were used, the two sets being treated separately.

COMPARISON OF AVERAGE TOTAL LEAF-PRODUCT VALUES FOR CLONS WITH CORRESPONDING VALUES FOR THEIR MOTHER PLANTS

For the five clons of the Howard 17 variety in 1925 the seasonal march of the total leaf surface per entire clon was compared with the corresponding march of the total leaf surface per mother plant in the clon, leaf-product values being used as indicators of leaf surface. The results are shown in Figure 9. The deviation from the average is seen to be small throughout the period, becoming larger for the later dates. The graph of the averages for mother plants alone is not far from the horizontal line for 1,500, showing again that the production of runner plants soon put a stop to the regular increase in net leaf surface of mother plants that was manifest for the first three intervals considered, beginning May 13, 21, and 27. The lined area between the two average graphs represents for each interval the portion of the total leaf product per clon that was due to leaves borne by the runner plants of the clon.

By July 8 the total leaf area of the runner plants of a clon nearly equaled that of the mother plant, and by August 20 the runner-plant portion of the clonal leaf area was nearly seven times as great as the mother-plant portion.

SUMMARY

This paper reports some of the results of a series of field studies on the relations between the growth of strawberry plants and the climatic conditions throughout the growing season. As an index of growth the leaf product (which is the sum of the products of the length and breadth for all leaflets on the plant) was used, and this was found to be a better growth-index than the leaf product for the terminal or for either of the lateral leaflets or for any linear dimension.

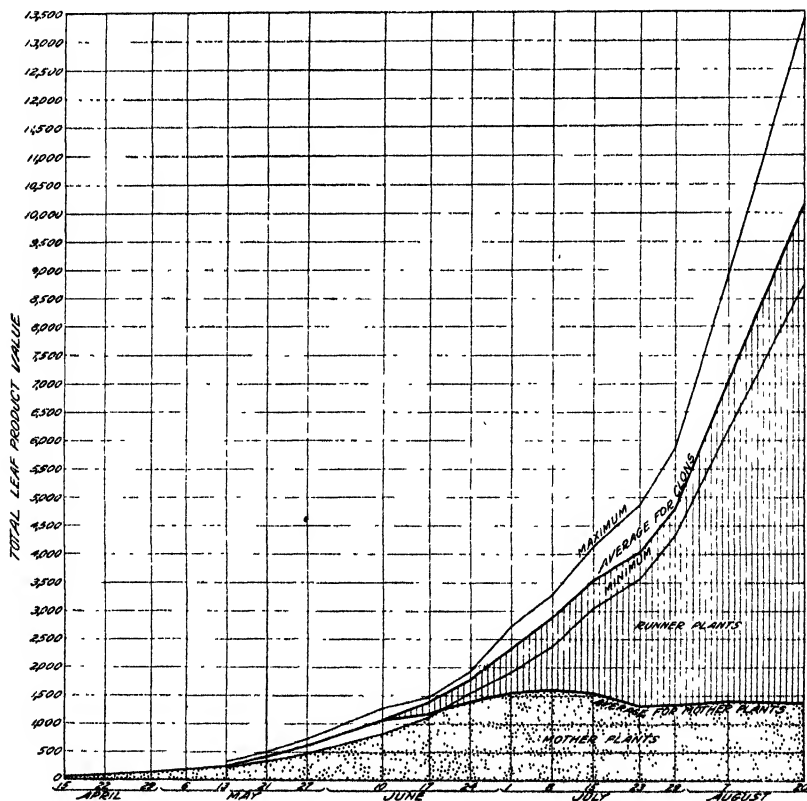


FIGURE 9.—Seasonal march of total leaf area per clon (as indicated by values of the total leaf product per clon) for five clons of Howard 17 variety, season of 1925; also the corresponding graph of the average total leaf product for the mother plant. The shaded area represents the runner-plant portion of the average total leaf product per clon at each time of observation

Measurements were taken at intervals, generally of one week, and seasonal graphs were constructed to represent the march of the leaf-product index throughout the season. Some of the climatic conditions were measured at like intervals, and graphs for these were also constructed.

A comparative study of these graphs with special reference to periods of high and low rates of leaf production leads to the conclusion that for the seasons 1925 and 1926 the limiting factor in the growth of the plants was generally temperature. In one instance, however,

low growth rates appear to have been due to drought. The temperature relations are considered in some detail, and the conclusion is reached that these data indicate high growth rates for average mean daylight air temperatures from about 68° to about 79° F., while daylight-temperature values above or below this range were generally accompanied by much lower rates of leaf production. These considerations lead to the approximation of a daylight-temperature optimum of about 73°.

It is emphasized that the results of these studies are to be regarded as valid only for the particular plants and seasonal and other environmental conditions that were involved in this work, and no generalizations are attempted.

Attention is called to the seasonal march of average mature-leaf size on spring-set plants with a seasonal maximum in June. Following the maximum, plants producing runners showed a decline in the size of their mature leaves for the remainder of the season, whereas plants with runners removed showed no such decline. This seasonal march for runner plants also showed an initial minimum, a later maximum, and a final decline. For the Howard 17 variety the average length of life of plants that unfolded between April 1 and July 8, 1925, was 56 days, with a range from 21 to 77 days. In general, the leaves died in sequence, those unfolding first being the first to die.

Plants allowed to produce runners showed a gradual increase in total leaf product until about July 8, after which this measure of leaf area decreased, but only to a slight extent for the rest of the period. By July 8 the total leaf product of the runner plants produced equaled that of the mother plants, and by August 20 it was about seven times as great.

A comparison of the seasonal march of the value of the average total leaf product per plant for 1925 for seven varieties shows that Howard 17 was the most active and Aroma the least active variety. Net leaf production by plants with runners removed was compared with that by plants with runners left on and allowed to root. The vigor of the parent plant was greatly increased by the removal of runners as they appeared, but the maximum deviation from the average was greater for these plants than for those that produced runner plants. A similar comparison was made between the mean daily increment values of total leaf product for the same plants. For plants with runners removed these values were all positive, although with much fluctuation; whereas for the plants producing runners they were generally much smaller and were actually negative (indicating a net loss) for 3 of the 17 periods.

A STUDY OF SOME UNPRODUCTIVE CHERRY TREES IN CALIFORNIA¹

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INTRODUCTION

Observations made in California over a period of years have disclosed an interesting problem regarding unproductive cherry trees. An occasional tree, or portion of a tree, has been found that produces practically no fruit, yet branches of the same tree, or other trees of the same variety standing beside it, have been consistently prolific. The behavior of these unproductive trees suggests the presence of a virus disease such as mosaic, but the results of the studies thus far conducted indicate that from a commercial standpoint they may be treated as a result of bud variation. The Black Tartarian is the only variety of cherry widely grown in California in which the occurrence of this unproductive strain is frequent. In orchards of this variety it is common to find 1 tree in 10 made up entirely or largely of the unfruitful type of wood, and much higher percentages have been noted. Occasional trees of the Bing, Lambert, Black Eagle, and other varieties have been found with leaves much like those of the unproductive Black Tartarian. Affected trees of these varieties have been found bearing considerable fruit, but it is misshapen and not marketable.

The conditions under which the productive and unfruitful Black Tartarian trees are growing appear to be identical, and in some instances the two types of trees are so close together that their branches overlap. (Fig. 1.) Trees of the unproductive strain have been found in all the orchards in central California where a search for them has been made, and they have also been found in southern California and northern Oregon. Not all cherry-growing localities in these latter districts have been searched carefully for affected trees.

The presence of 7 trees that were made up entirely or almost entirely of unproductive branches was recorded in one orchard in 1924, 17 in another orchard in 1925, and an additional 94 in a third orchard in 1926. In these orchards there are, respectively, 200, 190, and 980 trees of the Black Tartarian variety. A number of the trees that failed to fruit had been top grafted or removed from the first-mentioned orchard before these records were made. Many trees of the same type have been observed in other orchards. None of the affected trees or branches examined have improved and become productive, although the condition has become more noticeable on some trees with the growth of the affected branches.

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LEAF SYMPTOMS

The shape and color of leaves on the unproductive trees differ from those on productive trees, the blossoms are defective, and many blossom buds fail to open. "Curly leaf trees," "wild trees," and "male trees" are terms frequently used by growers to describe the unfruitful strain. The leaves produced by these trees have large or small areas that are somewhat dwarfed, and this dwarfed condition sometimes causes pockets or drawn places in the leaves which give them a wrinkled appearance. To one familiar with these irregularities, the affected leaves present an unnatural appearance even from a distance of two tree spaces. Affected leaves are distinctive in shape. (Fig. 2.) The margins are very irregular, due to the lack of development of



FIGURE 1.—Productive (right and left) and unproductive (center) Black Tartarian cherry trees. All three of the trees are of the same age and have been given the same treatment

some part of the leaf. This dwarfing may affect the end or part of the end of the leaf, the side or part of the side, or the entire leaf. The dwarfing may vary considerably in severity in different parts of the leaf. The dwarfed places range in size from small spots to areas that include almost the entire leaf. Sometimes they appear as narrow strips instead of spots and extend out from the midrib. Many leaves have more than one, and occasionally there are several dwarfed places in a single leaf. Sometimes only a small segment of the leaf fails to develop normally, causing a notch in the margin. This dwarfed condition is often more pronounced along one side of the leaf than the other, and sometimes one side fails almost entirely to develop while the other side appears to be normal.

In color the leaves of the unproductive trees differ from those of the normal leaf. With the unproductive tree there is a characteristic mottling, as the dwarfed areas are a lighter green than the remainder

of the leaf. The margins of the light-green areas are not always well defined around the entire periphery, and sometimes the spots fuse with one another or gradually disappear into the darker green portion of the leaf. These light-green areas are present when the leaf first unfolds in the spring, and they appear to result from the lack of development of chlorophyll rather than from discoloration. Outside the light-colored area the leaf is often a darker green than the normal Black Tartarian leaf. The two extremes of color and the dwarfing of the light-green areas of the leaf are sufficiently striking to enable an experienced observer to recognize the unproductive tree readily.

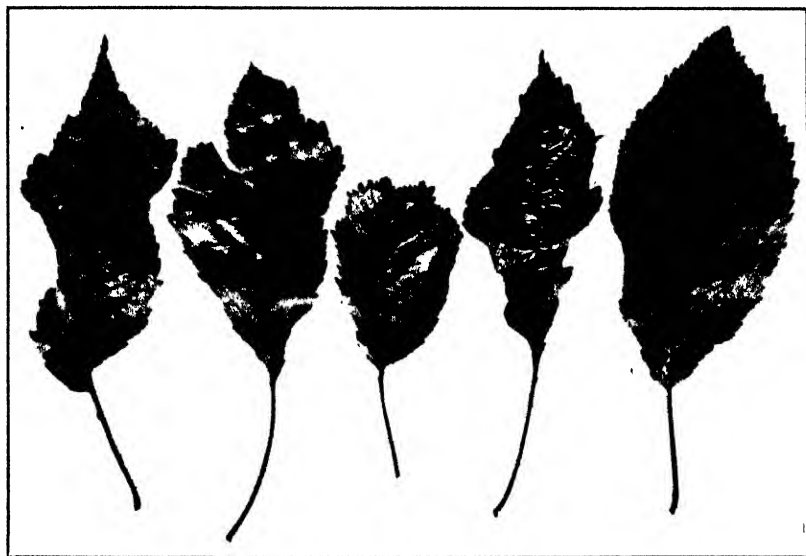


FIGURE 2 -- Four malformed leaves (left) from an unproductive Black Tartarian cherry tree and one normal leaf (right) from a thrifty tree in the same orchard

The failure of the leaf, or a part of it, to reach normal size results in a much lower average leaf area on the unfruitful trees than on the normally fruitful ones. Measurements of leaves taken from a 15-year-old tree which has borne almost no fruit and where the malformation is thought to be about average for unproductive trees show that the average leaf area is only three-fourths that of a normal tree standing beside it. On some of the less vigorous of the unproductive trees the leaves appear to roll more during dry weather than those of productive trees, while leaves of the more vigorous ones appear to roll less. The effect of drought on the leaves seems to be determined rather by the vigor of the tree than by the trouble that causes the abnormal development of the leaves.

The extent of mottling and the irregularity in the shape of the leaf of the unproductive strain are sometimes less pronounced on the leaves of shoots that are produced during the summer than on those grown from the buds that open in the spring. Instances have been noted where leaves that grew during the summer were affected but

little if any, whereas all leaves of the same tree that grew early in the spring and in the spring following were decidedly abnormal.

At least an occasional affected leaf was found on the majority of the Black Tartarian trees examined, although on many of these trees no other trace of the malady was apparent. Sometimes the affection was limited to only a few leaves or to the leaves on an occasional spur or branch, and in other cases it was displayed over much of the tree. On the so-called unproductive trees normal leaves and normal fruits could also be found. Some Black Tartarian trees appeared to be entirely free from the off-type leaves.

GROWTH AND BLOSSOMING

In orchards where observations have been made, including those not yet in bearing and others up to more than 20 years of age, there appears to be no difference in the rate of growth between the normal and the unproductive trees. Trees of both types standing side by side have been observed, but no consistent difference in size of trees of



FIGURE 3—Exposed pistils of blossom clusters of productive (one at right) and unproductive Black Tartarian cherry trees

the same age has been noted. Occasionally a tree of the unproductive strain is larger than its neighbor, and sometimes smaller, but usually it is about the same size. In one orchard, which contains 190 trees of the Black Tartarian variety, the largest tree is made up mostly of affected branches.

The unfruitfulness of the trees under discussion is not due to their failure to blossom, for they bloom profusely and at the same time as the productive trees. Many of the blossoms are defective, however, and smaller than those of normal trees. The peduncles are short, the petals small, and the pistils short and slender with a tendency to discolor early. Many of the pistils fail almost entirely to make any growth. (Fig. 3.) Among the discolored pistils some turn brown before the blossoms open, some soon afterwards, while some retain their normal color for a few days. Now and then a pistil is observed that appears to develop like those on fruitful trees. An occasional fruit is found on unproductive trees, but it is small, rough, and ill shaped. (Fig. 4.)

Although the unfruitful trees blossom profusely, some of the blossom buds that are formed fail to open. The buds at and near the terminals of spurs and shoots produce their blossoms, while those nearest the base of the previous year's growth often fail to open fully. (Fig. 5.) These buds usually swell but stop growth about the time the scales open and the green-bud clusters appear. The swollen buds turn brown and become dry, but often remain in place on the shoot or spur for several weeks.

SOIL AND CULTURAL CONDITIONS

In some orchards where unproductive trees have been under observation there is considerable variation in thrift and production among the trees due to soil or cultural conditions, but in none of the cases observed can the peculiarities of the unproductive trees be attributed to these causes. In some orchards the accumulation of soil moisture during part of the year is responsible for considerable variation in the

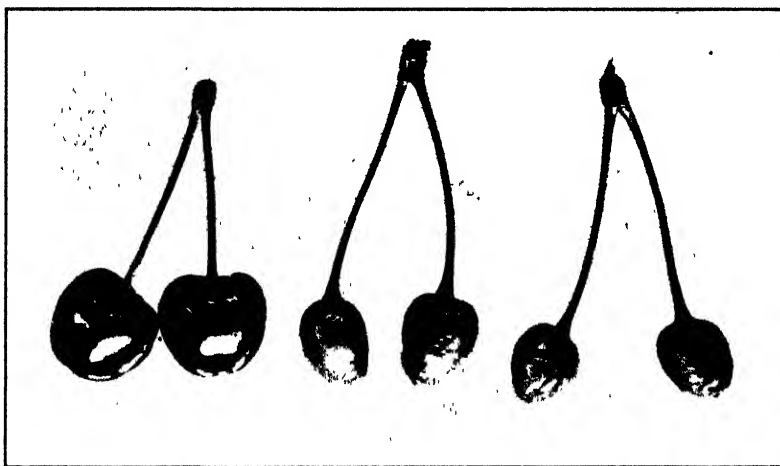


FIGURE 4.—Clusters of cherries from normal (left) and unproductive (two at right) Black Tartarian cherry trees

thrift of the trees. In these orchards a stratum of hardpan underlies the surface soil at a depth of from 2 to several feet, preventing a free filtration of water. This results in an accumulation of soil moisture during the winter months when rainfall is usually plentiful, and occasionally also after summer irrigations. In these places where conditions for growth are unfavorable some trees have died, others have made but little growth, and the yield of fruit and size of leaves have been below normal. The peculiarities of the unproductive trees under discussion are not, however, such as are found in these wet places, nor are they the symptoms commonly found in soils unsuitable to root growth. Trees of the unproductive strain are not more numerous in such places than where satisfactory conditions for growth prevail.

In a study of the distribution of unproductive trees throughout the orchards there was no indication that the local environment was responsible for their behavior. A few of these trees were found more or less grouped in different parts of the orchards, but as frequently

they were scattered singly over large orchards. In some orchards only an occasional unproductive tree was found; in others they were numerous.

In a few of the orchards under observation a number of trees have been injured to such an extent by the application of stable manure that many of their branches have died. The annual growth on these trees has been very short, and the leaves are small. Many of the leaves on the upper and outer branches are light-green or yellowish in color. The unthrifty appearance of these trees differs, however, from that of the unproductive strain, and none of the abnormal characteristics of the unfruitful trees are found on those injured by the manure. Some of the unproductive trees studied are in orchards that have been given manure, and others are in orchards that have received none.



FIGURE 5.—A branch of an unproductive Black Tartarian cherry tree on which the flower buds below the terminals of the shoots and spurs failed to open

EFFECT OF TOP GRAFTING AND BUDDING

A number of trees of the unproductive strain which were recognized by growers have been either removed or top grafted with scions from productive trees, both of the Black Tartarian and of other varieties. This explains why there are not more of them found in some Black Tartarian orchards. In all instances observed the new tree tops which followed grafting have been normally productive and free from any of the malformation of leaves, blossoms, or fruit common to the off-type trees. Where shoots have been allowed to grow from the portion of the branches that remained after the tree was headed back for grafting, the leaves produced by them have manifested the same irregularities in shape and color that were found on the tree before it was headed back, although the growth from the scions has been healthy and productive. Shoots which grow from recently cut-back unproductive trees make a rampant growth, and their leaves develop to a large size, but this has not prevented the development of the characteristics of the unproductive tree in them.

For experimental purposes a few trees of the unproductive strain of the Black Tartarian variety were top grafted in 1928 with scions from both productive and unproductive Black Tartarian trees, both types of scions being inserted into the same tree. Several branch stubs in each of a number of trees were grafted in this way. As the scions grew all those from the productive trees produced normal leaves, and all those from the unproductive trees displayed the same characteristics as the tree from which they were taken. Neither in their first nor second year's growth have the leaves of any of the scions changed in their habit of growth, but they are the same in appearance as those of the parent trees.

In the spring of 1929 the scions from productive trees produced numerous blossoms, all of which appeared to be normal and set fruit of normal shape which grew to maturity. Many of the flower buds of the scions from the unproductive trees failed to open, and many of the blossoms which opened were small, had short unthrifty pistils, and none of them set fruit. A number of branches were allowed to remain on the top-grafted tree when its branches were cut back for grafting; and as the trees were headed back at a height of about 5 feet, numerous small branches and spurs are still growing on the stock portion of the tree. Neither the pruning done in heading the tree back nor the top grafting has affected the habits of these remaining branches. They are unproductive, and the leaves and blossoms are of the same unthrifty type as those produced before the branches were headed.

Attempts were made to transmit the faults of the unproductive strain by budding. Buds from this strain of Black Tartarian were inserted in nursery seedlings in the spring of 1928. The growth from these buds exhibited the same faults as the trees from which the buds were taken, while that on the check trees was normal.

UNSUCCESSFUL ATTEMPTS AT TRANSMISSION BY OTHER MEANS

Efforts to transmit by other means the abnormal development in unfruitful trees have been unsuccessful. Branches of fruitful and unfruitful trees growing side by side have been fastened together in such a way that the leaves would rub one another as the branches were moved by the wind; juice from affected leaves of different ages has been expressed and immediately applied to healthy leaves of different ages, some of which had been pierced and some just slightly scratched to break the epidermis; healthy and affected leaves on adjoining branches have been pinned together and left remaining on these branches; but no indication has been observed that any of the leaf symptoms that accompany unproductiveness have been transmitted.

A number of instances where fruitful and unproductive branches are growing side by side on the same tree have been under observation for three successive years. (Fig. 6.) During this period the performance of none of the branches has changed. The unproductive branches, with their unthrifty leaves, have remained the same, while the productive branches have been healthy and prolific. There is no indication that the position of the branches on the tree influences their behavior.

The occurrence and prevalence of off-type leaves and unfruitful branches in the varieties of cherries studied resembles the condition found in some of the common variegated plants on which occasional normal leaves, twigs, and branches occur.



FIGURE 6.—Unproductive (left) and productive (right) branches growing side by side on the same Black Tartarian cherry tree

PREVENTIVE AND REMEDIAL MEASURES

From the observations thus far made it appears that the unproductive trees are a strain of a variety that has resulted from the propagation of "sporting" branches or "variegations," and if such is the

case the trouble may be evaded by care in the choice of propagating wood. Since the irregularities in the leaves are less noticeable in the fall than earlier in the season, and leaves of shoots that are produced during the summer often display only a trace of the trouble, the location and labeling of propagating wood may be most surely and easily done before the fruit is harvested.

Whatever may be the cause of the trouble that results in the failure of the trees to fruit, the removal, by pruning, of affected branches and by top grafting where the entire tree or a considerable portion of it is affected are effective remedial measures. The selection of propagating wood from productive trees appears to be a satisfactory preventive measure.

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE TOXICITY OF NICOTINE, PYRIDINE, AND METHYL-PYRROLIDINE TO MOSQUITO LARVAE¹

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INTRODUCTION

Previous investigations of the toxicity to insects of nicotine in aqueous solution have involved principally a study of the effects of its application as a spray or dip. The solutions employed have generally contained nicotine sulphate and soap or other alkaline substance, the purpose of the latter being to increase the wetting and spreading properties of the liquid and to convert the nicotine salt into the uncombined, volatile base. After the application, the insect is in contact with continuously varying quantities of nicotine in solution and nicotine gas as evaporation of the alkaloid and water progresses. Under these conditions the toxic effect of nicotine has been explained as the result of its entrance in the gaseous state through the spiracles into the tracheal system of the insect.

In addition to the variable concentration, the results of treating insects by the method just mentioned are complicated by the presence of the alkaloid in three conditions, viz, as gas molecules, as molecules in solution, and as ions. It seemed probable that additional light could be thrown upon the nature of the effect of nicotine upon insects if gaseous nicotine could be eliminated, leaving only the molecules and ions in solution to act upon them. Then, by varying the hydrogen-ion concentration, the insects could be subjected to solutions differing widely in relative content of dissolved molecules and ions.

Accordingly, an aquatic insect, the larva of the house mosquito (*Culex pipiens* L.) was chosen for the purpose and proved to be an excellent test insect.

EXPERIMENTAL PROCEDURE

MATERIALS AND METHODS

The mosquito larvae were reared in a greenhouse in battery jars 15 cm. in diameter. Each of the jars contained approximately 3 liters of tap water in which a quantity of soil and decayed leaves was suspended. Within 24 hours most of the solid matter fell to the bottom of the jars, leaving a solution rich in food for the larvae. Female mosquitoes entered the greenhouse on warm nights and oviposited freely on the surface of the water in the jars. Each morning the egg boats were collected and distributed among the jars so that each would contain the desired number of larvae of about the same age. The experiments extended from July 1 to November 1, 1927.

¹ Received for publication Mar. 6, 1930; issued September, 1930.

² Resigned June 20, 1928.

All nicotine solutions used were made up to the desired molar concentration by diluting 96 per cent nicotine with distilled water. The solutions of nicotine sulphate and nicotine hydrochloride were adjusted to the desired hydrogen-ion concentration with 0.1 normal sulphuric or 0.1 normal hydrochloric acid solutions, respectively, and then diluted. The pH values were determined colorimetrically and checked by the hydrogen electrode, using a saturated calomel half cell, a saturated potassium chloride salt bridge, and a Clark shaking hydrogen electrode assembly with platinum electrodes. The values obtained by the two methods agreed within about 0.1 pH, the electrometric values always being higher.

A group of 10 active, feeding larvae in the last instar was used for each experiment except the tests with 0.1 molar solutions in which the time of action was very short. The larvae were removed from the culture to a small dry beaker by means of a medicine dropper with a wide orifice. The culture solution carried over with the larvae was then removed until not more than about 0.1 c. c. remained in the beaker. Thirty-five cubic centimeters of the nicotine solution at the temperature of the water bath were gently poured into the beaker, which was then placed in a water bath maintained at $26 \pm 1^{\circ} \text{C}$. A few experiments were made at slightly lower temperatures, but the limits for the entire series fell between 24° and 27° . The volume of the solution stated above was not rigidly adhered to, and sometimes was as low as 12 c. c. Since 10 larvae of the size used in these experiments weigh about 32 mgm., the quantity of nicotine solution was always very large as compared with the mass of the larvae.

The time to the nearest second from the immersion of the larvae in the nicotine solution to the time the fifth one of the 10 became immobile was taken as the time of action of the particular nicotine concentration for 50 per cent of the larvae in each experiment. The action of 0.1 molar nicotine solution was so rapid that it was necessary to reduce the number of larvae in each experiment to four.

THE PROCESS OF POISONING

The course of poisoning of *Culex* larvae in a nicotine solution requires some consideration at this point. In a 0.03 molar solution the larvae swim about for a brief period, making few excursions to the surface. Soon, however, they fall writhing to the bottom of the beaker, and at times make short, vigorous, uncoordinated swimming movements. Movement gradually subsides, and the body then stiffens into a state of comparative immobility. This was chosen as the end point, and it is quite decisive to an experienced worker. Slight twitching, especially of the siphon, still occurs in response to gentle pressure with a needle, the dorsal heart continues to pulsate, and movements of the digestive tract are visible through the translucent body wall.

The effects of other concentrations differ essentially from the one just described only in the time of appearance of the characteristic phenomena. Life continues for a long, but variable, period following the beginning of this immobile stage. It is manifested by feeble body movement, irregular heart action, and movement of the digestive tract. Preliminary experiments have shown a certain degree

of recovery following transference to water after the first immobile stage is reached, but a quantitative study of it has not yet been completed.

Theoretically a compound like nicotine may enter the body of a submerged *Culex* larva by at least four routes: (1) Through the siphon and thence into the tracheal system; (2) through the tracheal gills on the anal segment into the tracheal system; (3) through the cuticula into the tissues and blood; (4) through either the mouth or anus into the digestive tract.

The siphon opening of the *Culex* larva is guarded by five petallike valves which are usually held tightly together when the larva is submerged but which open when the tip of the siphon is pressed against the surface film as the larva seeks a fresh supply of oxygen. Although Wesenberg-Lund (24, p. 21)³ observed that the valves are not always completely closed when the normal larva is submerged in water, frequent observations made by the writers showed that it was closed when the larva was placed in the nicotine solution. It is therefore believed that little if any nicotine, either as gas or in solution, enters the body through the siphon.

Structurally, the tracheal gills appear to offer an excellent opportunity for the passage of substances dissolved in water. However, no evidence that the gills are permeable to nicotine was obtained from this study.

The chitinous cuticula of the larva is probably at most slowly permeable to nicotine. Indirect evidence of its impermeability to this compound is furnished by experiments with *Culex* pupae, which survive for long periods (20 hours or more) in nicotine solutions (0.003 M and 0.012 M) and may even transform to adults before death. The pupae have no mouth opening. In view of the lack of evidence to the contrary, the writers believe that the toxic effects of nicotine in solution produced in *Culex* larvae are not due to the passage of the compound through the chitinous cuticula.

When first placed in a weak solution of nicotine the larva swims about, making typical feeding movements with the rotary mouth brushes. As soon as the effects of the poison become apparent, the larva opens the mandibles widely. Under these conditions it appears certain that the nicotine enters the digestive tract in quantities sufficient to produce toxic effects. Entrance through the anus is also a possibility, but evidence in support of it is lacking.

From a consideration of the structure of the larvae and their behavior in the solutions, it is probable that nicotine enters the body chiefly through the mouth as molecules or ions in solution rather than as molecules in the gaseous condition. Bodine (1) concluded that certain acids and mercuric chloride penetrate mosquito larvae orally rather than cutaneously.

EXPERIMENTAL RESULTS

THE TOXICITY OF NICOTINE AND NICOTINE SULPHATE SOLUTIONS AT VARIOUS pH VALUES

Nicotine solutions of 0.03 molar concentration, titrated with 0.1 normal sulphuric acid solution to pH values from 2.4 to 7.0, were used in a series of experiments on mosquito larvae. The results are

³ Reference is made by number (italic) to Literature Cited, p. 347.

summarized in Table 1, and are plotted in Figure 1 as reciprocals of the time in seconds to immobility of 50 per cent of the larvae. The toxic effect of the solutions changes at first slowly with rising pH value, then more abruptly, attaining a maximum for the solution of highest pH value, i. e., nicotine base. Similar results were obtained with solutions titrated to the desired pH with hydrochloric acid solution (Table 1), indicating that the toxicity of the various solutions was not due to a specific action of the sulphate ion.

TABLE 1.—Toxicity of nicotine, nicotine sulphate, and nicotine hydrochloride solutions at various pH values to the larvae of *Culex pipiens* in 30 groups of 10 larvae for each test, 1927

Compound	Molar concentration of nicotine	pH	Dates of experiments	Mean seconds to immobility of 50 per cent of larvae ^a	Coefficient of variation ^b
Nicotine sulphate	0.03	2.4	Sept. 16, 20.	2,425±65	14.7
Do.	.03	3.6	Sept. 17, 20.	2,122±78	20.1
Do.	.03	5.0	Sept. 14, 20.	1,360±43	17.3
Do.	.03	7.0	Aug. 16, 17; Sept. 21.	507±37	39.6
Nicotine	.03	9.7	Sept. 13, 15, 21.	321±15	24.9
Nicotine hydrochloride	.03	3.6	Oct. 27, 28, 29.	1,882±77	22.4
Do.	.03	5.0	Oct. 4, 5, 20.	1,363±49	19.5

^a The values for the error of the mean (σ_M) were computed from the formula: $\sigma_M = \frac{\sigma}{\sqrt{N}}$, in which σ is the standard deviation and N the number of groups of larvae.

^b The coefficient of variation is the ratio (σ mean seconds to immobility) multiplied by 100.

The curves for toxicity of nicotine to mosquito larvae and for the dissociation of nicotine are compared in Figure 1. The dissociation constants for a 0.01 molar solution at 15° C. and the pH values of the neutral and basic salts of nicotine are taken from Kolthoff (9, 10). Nicotine is a weak diacid base. The first dissociation constant is 7.07×10^{-7} ($pK_1 = 6.16$); the second dissociation constant is 1.12×10^{-11} ($pK_2 = 10.96$). The first dissociation apparently involves the nitrogen of the pyrrolidine ring, the basic salt having a pH value of 5.6 whereas the second dissociation involves the nitrogen of the pyridine ring, the pH value of the neutral salt being 2.6. The pK value for pyridine, as given by Kolthoff, is 8.90.

An inspection of Figure 1 shows a close relationship between the first dissociation curve for nicotine and the curve for toxicity—both the percentage of dissociation and the speed of toxic action, expressed in reciprocals of the time in seconds elapsing until the larvae become immobile, being plotted against pH. The second dissociation, however, affects the shape of the toxicity curve somewhat, although to a very much less extent than does the first dissociation. As a result, in the region of the pH value of the basic salt (pH 5.6) toxicity approaches its minimum value.

These curves are believed to demonstrate two propositions: (1) That the speed of entrance of nicotine from an aqueous solution into *Culex* larvae is related to the concentration of undissociated nicotine base in the solution; (2) that in so far as the toxicity is concerned with the ionization of the nicotine molecules, it is governed largely by the dissociation of the pyrrolidine nitrogen. The latter statement is made on the assumption that the first dissociation of nicotine

must be governed by the pyrrolidine nitrogen in the same way that the second dissociation is governed by the dissociation of the pyridine nitrogen. The pK value of methylpyrrolidine has apparently not been determined but will probably be found to be less than 6.

THE TOXICITY OF NICOTINE AND NICOTINE SULPHATE SOLUTIONS AT DIFFERENT CONCENTRATIONS OF NICOTINE

Nicotine solutions of 0.1, 0.03, 0.01, and 0.001 molar concentrations were each titrated to pH 5.0 with 0.1 N sulphuric acid solution and compared in toxicity with solutions of the base at the same molar concentrations. The results are given in Table 2 and Figure 2.

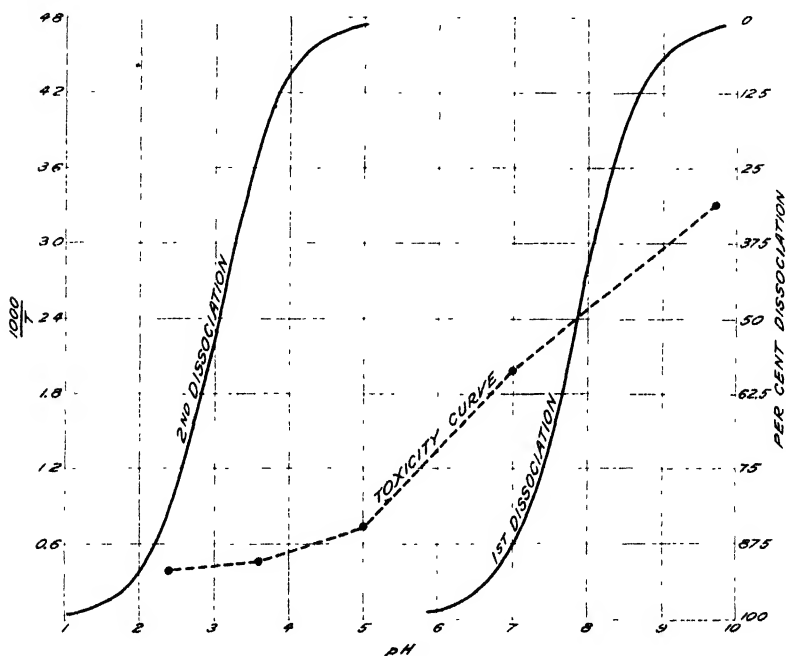


FIGURE 1.—Toxicity to the larvae of *Culex pipiens* of 0.03 M nicotine at various hydrogen-ion concentrations compared with the dissociation of nicotine. The toxicity is plotted in terms of the reciprocals of the time T , to the seconds needed to produce immobility in the larvae. The dissociation curves are taken from the works of I. M. Kolthoff (9, 10). This figure shows the two series of data by superimposed graphs. The figures for the toxicity curve are shown at the left of the chart and the percentages for the dissociation curves at the right.

The speed of toxic action of the nicotine base, expressed as the reciprocal of the time in seconds to immobility, follows nearly a straight line between the concentrations of 0.01 and 0.1 molar. In this range the proportion of nicotine ions falls from about 3 to less than 1 per cent. The increase in toxicity with increasing concentration is therefore chiefly due to the effect of the content of undissociated nicotine hydroxide molecules rather than to the nicotine-ion content. In the solutions containing nicotine sulphate (pH 5.0), more than 99 per cent of the molecules are in the ionic state, and the speed of toxic action for them at every concentration is much less than for the solutions of the base having the same nicotine concentration. When the ratio (seconds to immobility in the nicotine

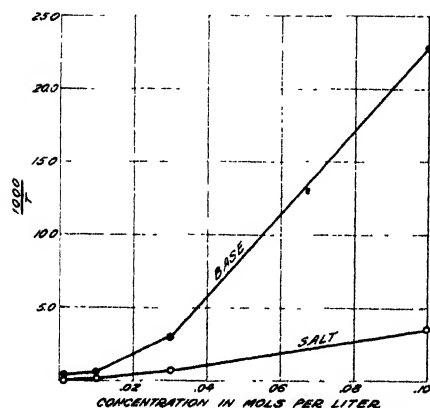
TABLE 2.—Toxicity of nicotine and nicotine sulphate solutions to the larvae of *Culex pipiens* at various base concentrations

[Nicotine sulphate solutions adjusted to pH 5.0]

Compound	Molar concentration of nicotine	pH	Dates of experiments	Number of larvae in each group	Number of groups	Mean seconds to immobility of 10 per cent of larvae	Coefficient of variation ^a	Mean ratio of toxicities of base and salt
Nicotine	0.1	9.95	Oct. 8, 10, 11, 21, 24, 26.	4	49	4 ^a ±1.8	29.3	5.7
Nicotine sulphate	.1	5.0	Oct. 5, 6, 7, 21, 24, 26.	4	49	281±14	35.9	
Nicotine	.03	9.7	Sept. 13, 15, 21.	10	30	321±15	24.9	4.5
Nicotine sulphate	.03	5.0	Sept. 14, 20.	10	30	1,350±43	17.3	
Nicotine	.03	9.7	July 20, 21, 22, 23, 24, 27, 28, 29, Aug. 2.	1	200	176±5	43.8	6.8
Nicotine sulphate	.03	5.0	July 23, 25, 26, 27, 28, 29, Aug. 1, 2.	1	200	992±51	72.9	
Nicotine	.01	9.6	Sept. 7, 8, 9, 10.	10	20	1,622±54	14.9	6.2
Nicotine sulphate	.01	5.0	Sept. 9, 10.	10	20	9,783±142	6.7	
Nicotine	.001	8.5	Sept. 1, 6.	10	20	4,056±309	34.1	5.2
Nicotine sulphate	.001	5.0	Sept. 7, 8.	10	20	25,200	—	

^a The coefficient of variation is the ratio (σ , mean seconds to immobility) multiplied by 100.^b Estimated.

sulphate to seconds to immobility in the base) is calculated for each group of larvae—the groups in each series of tests being arranged in pairs in chronological order of the experiments—the mean of all of the ratios tends toward values between 5 and 7; i. e., nicotine base at the concentrations used was about 5 to 7 times more toxic than nicotine sulphate at pH 5.0. This result was obtained with individual larvae as well as with groups of 10 larvae. (Table 2.)

FIGURE 2.—Comparison of the toxicity of nicotine and nicotine sulphate (pH 5.0) to the larvae of *Culex pipiens* at different molar concentrations. The toxicity is plotted in terms of the reciprocals of the time, T , in seconds needed to produce immobility of the larvae.

THE EFFECT OF SUPPRESSING THE IONIZATION OF NICOTINE BY THE ADDITION OF HYDROXYL IONS

Since an increase in the concentration of unionized nicotine increases the toxicity of nicotine solutions, the question arises if toxicity can also be increased when the ionization of the nicotine base is suppressed by the addition of hydroxyl ions. However, the nicotine base is so slightly ionized that suppression of the ionization would be expected to yield very little additional toxicity. For instance, a solution containing 0.03100 mols of the nicotine base per liter theoretically contains 0.03085 mols of unionized nicotine per liter, solutions of 0.01000 molar strength contain 0.00992 mols of unionized nicotine per liter, and those of 0.001000 molar strength contain 0.000974 mols of unionized nicotine per liter. When these solutions are made to contain 0.01 mols of sodium hydroxide per liter the ionization of nicotine may be said to be completely suppressed, since in any of these

solutions there was less than 1 part in a thousand of nicotine that remained ionized. The suppression of ionization by the sodium hydroxide, therefore, only affected 4.7 parts in a thousand in the case of 0.031 molar nicotine, 8.4 parts in that of 0.01 molar nicotine, and 26 parts in that of 0.001 molar nicotine.

Solutions of 0.001 M, 0.01 M, and 0.03 M nicotine were made up to contain from 0.001 M to 0.05 M sodium hydroxide, the excess of hydroxyl ions being sufficient to suppress almost completely the ionization of the nicotine. The results are expressed in Figure 3 as ratios of the time in seconds needed to bring about immobility in nicotine solution containing sodium hydroxide to time in seconds to the same effect in nicotine alone.

Solutions of sodium hydroxide of a concentration of 0.05 M or less are comparatively nontoxic to *Culex* larvae, the larvae dying only after exposure to such solutions for from 2 to 12 hours.

The results with 0.03 M and 0.001 M nicotine show clearly that a further suppression of ionization of the nicotine leads to no significant change in toxicity, the ratio, 1.0, being closely approached in both cases. The values obtained for 0.01 M nicotine are, with the one exception shown, more irregular.

A series of experiments in which barium hydroxide, calcium hydroxide, and potassium hydroxide were substituted for sodium hydroxide gave toxicity values in good agreement with those for sodium hydroxide.

The conclusion reached may be stated as follows: In aqueous nicotine solutions of 0.001 M concentration or higher, the addition of an inorganic base increases the concentration of unionized nicotine so slightly that under these conditions no increase in toxicity can be observed.

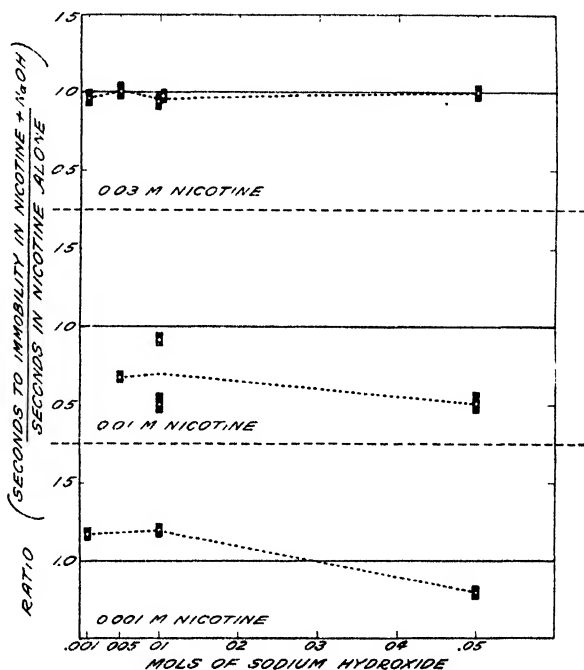


FIGURE 3.- Relation of the suppression of ionization of nicotine by sodium hydroxide to the toxicity to larvae of *Culex pipiens*. Along the ordinate are indicated ratios of seconds needed to produce immobility in the larvae in nicotine with sodium hydroxide to time in seconds to produce the same in nicotine alone. Along the abscissa are indicated mols of sodium hydroxide added to the nicotine solutions.

THE TOXICITY OF PYRIDINE AND METHYLPYRROLIDINE

Pyridine solutions of 0.03 and 0.12 molar concentrations were titrated to pH 5.0 and 4.9, respectively, with 0.1 normal sulphuric acid, and their toxicities to *Culex* larvae were studied in comparison with solutions of pyridine base of the same concentration. A few experiments were also made with a small sample of methylpyrrolidine,⁴ the concentrations being 0.03 M, the pH values for the base approximately 9.8, and for the salt (hydrochloride) 3.0.

The results are given in Table 3. They show clearly that these compounds, components of the nicotine molecule, are more toxic as bases than as salts. Both compounds, either as bases or salts, are strikingly less toxic than nicotine, indicating that the toxicity of nicotine results from something inherent in the combination of pyridine with methylpyrrolidine rather than in either of its components.

TABLE 3.—Toxicity of pyridine and methylpyrrolidine (base and salt) to the larvae of *Culex pipiens*

Compound	Molar concentration	pH	Dates of experiments	Number of larvae in each group	Number of groups	Mean seconds to immobility of 50 per cent of larvae	Coefficient of variation ^a	Mean ratio of toxicities of base and salt
Pyridine	0.12	7.8	Aug. 10, 11	1	40	702±26	23.5	2.2
Pyridine sulphate	.12	4.9	Aug. 12	1	40	1,566±56	22.6	
Pyridine	.03	7.1	Nov. 1	10	10	2,048±81	12.5	2.2
Pyridine sulphate	.03	5.0	Nov. 3	10	10	4,432±34	2.4	
Methylpyrrolidine	.03	9.8	Nov. 4	10	4	2,895±106	7.3	18.6
Methylpyrrolidine hydrochloride	.03	3.0	Nov. 4	10	6	54,000	—	

^a The coefficient of variation is the ratio (σ : mean seconds to immobility) multiplied by 100.

^b Estimated.

DISCUSSION

The idea that nicotine is more effective against insects than its salts seems to be an old one. In 1900, Del Guercio (6) reported some experiments in which silkworms (*Bombyx mori* L.) were sprayed with aqueous solutions of nicotine, nicotine containing alkali, and nicotine containing acid. No differences were noticed in the effects produced by the solution of nicotine and the nicotine containing alkali, but the acidulated nicotine was less active than the other solutions. Vermorel and Dantony (23, p. 23) added sodium carbonate to spray solutions containing a nicotine salt, explaining that its purpose was to liberate the nicotine from the combination. They further stated that it has been established for a long time that free nicotine is more toxic than nicotine salts. Hollister (7) submerged bedbugs (*Cimex lectularius* L.) in solutions of nicotine and nicotine sulphate of various concentrations, and claimed a slightly higher toxicity for the nicotine solutions. McIndoo (15) published the first extensive work on the action of nicotine as an insecticide. He concluded that nicotine spray solutions neither pass into the tracheae nor penetrate the integument, but that nicotine vapor enters the body through the tracheae and passes thence to the tissues. He employed solutions of nicotine and of nicotine sulphate but reported

⁴ The sample of methylpyrrolidine was kindly furnished by F. B. La Forge of the Insecticide Division, Bureau of Chemistry and Soils.

no difference in their action on insects. Moore and Graham (17) compared the effect of a solution of nicotine sulphate with a nicotine sulphate solution rendered alkaline with sodium carbonate upon the aphid *Macrosiphum sanborni* Gillette, the solutions being applied as sprays. The nicotine solution containing sodium carbonate was considerably more active than the nicotine sulphate solution without this addition. The results were held to be due to the presence of nicotine in volatile form in the alkaline solution. In a later paper (18) the same writers, confirming McIndoo's results, showed that nicotine solution does not penetrate into the tracheae but that nicotine vapor does enter them. De Ong (4, 5) sprayed and fumigated aphids with 0.1 per cent solutions of nicotine sulphate of various pH values from 6.5 to 8.2. The results from the two methods of application were parallel, the toxicity increasing with the increase of the pH value of the solutions. He believes that nicotine is a tracheal rather than a true contact insecticide. Worthley (25) has recently confirmed previous work in showing that alkalis increase the toxicity of nicotine sulphate solutions.

A few references to the relative toxic effect of nicotine as a base and as a salt on other animals than insects have been found in the literature. In 1889, Langley and Dickinson (12, p. 426) noted that if a nicotine solution were neutralized with sulphuric acid solution its effect both upon nerve fibers and upon ganglion cells was lessened. No explanation of these observations was attempted. Moore and Row (16) studied the comparative physiological action of nicotine, piperidine, and coniine upon the frog. They found that the free bases were more active than the respective salts, 2 mgm. of basic nicotine being as active as 10 mgm. of nicotine hydrochloride. De Ong (5) showed that free nicotine, administered orally, was very much more toxic to chickens than nicotine sulphate.

Studies on the toxicity of other alkaloids and similar compounds in relation to the reaction of the dissolving medium have been numerous, dating back at least as far as 1897, when Overton (19, p. 208) showed that ammonia, various amines, and many alkaloids enter cells readily as free bases, whereas the salts of these compounds if dissociated do not penetrate cells to a noticeable degree. Crane (3) found that the toxicity of alkaloids to *Paramoecium caudatum* varied with the dissociation constant of the base and stated that the free, undissociated base is apparently responsible for the toxicity. No evidence of a direct action of the hydrogen ions upon the resistance of the cell was obtained. Labes (11) studied the effect of hydrogen-ion concentration upon the toxicity of certain alkaloids and acids to bacteria and frog larvae and concluded that the undissociated, lipid-soluble base was more toxic than the lipid-insoluble ions. Copeland and Notton (2) found that the action of a local anaesthetic depends upon the specific selective affinity of its base for nerve fibrils and that the different salts of these compounds vary in their action according to the degree of dissociation. Anaesthetic action is relatively high when the base is combined with a weak acid, the solution having a high pH value, and is relatively low when it is combined with a strong acid, the pH value then being lower. Trevan and Boock (22) have studied the relation of hydrogen-ion concentration to the action of a number of local anaesthetics on the rabbit's cornea. The results are held to be consistent with the view that the free base is the only

active constituent of the solutions, the ions or undissociated salt molecules taking no part.

The investigations of the writers referred to above indicate, in general, that nicotine base is more toxic than nicotine salt when administered to insects as a spray or dip, and that it is more toxic to vertebrates as the free base when administered either by mouth or by injection. The results of studies on other alkaloids and organic bases, in which a variety of organisms was used, are in accord with those on nicotine.

The greater insecticidal effect of nicotine as compared with nicotine sulphate has been attributed to the action of nicotine gas molecules which are able to enter and diffuse through the tracheal system. The present studies show, however, that the free base in aqueous solution may also be more toxic to insects than the salt.

It should be noted that nicotine solutions of pH 2.4 still possess an appreciable toxicity although less than that of the free base. This apparently signifies that the ions are, in themselves, somewhat toxic, for in solutions having the concentrations of those used in this study ionization of the nicotine salt molecules is nearly complete.

Although the penetration of gases into living cells is a familiar phenomenon (cf. Jacobs, 8, p. 133), it seems doubtful whether nicotine can long remain in this state within the tracheal system of an insect because of its great affinity for water. It therefore probably enters the cell as molecules or ions in solution. Lillie (13, 14), working with certain organic acids which produce their maximum effect as undissociated molecules, holds that the acid molecules penetrate cell membranes rapidly and dissociate within the cell, yielding acid ions. Taylor (21, p. 218), who also worked with acids, believes that the hydrogen ions of the acid are adsorbed into the cell membrane, and the electric charge on the membrane then attracts the acid anion, the pair of ions gradually passing into the cell. It is not impossible that nicotine reacts with certain constituents of cells. The recent work of Petrunkin and Petrunkin (20, p. 108), in which they express the belief that certain alkaloids and organic bases combine with gelatin and brain proteins only on the alkaline side of the isoelectric point, are of interest in this connection.

SUMMARY

The toxicity of nicotine to the house mosquito (*Culex pipiens* L.) was studied in aqueous solutions at various pH values. A few similar experiments were also made with pyridine and methylpyrrolidine.

Solutions of 0.03 M concentration adjusted to pH values of 2.4, 3.6, 5.0, and 7.0, with sulphuric acid and at pH 9.7 (the free base) showed a toxic action that increased with increasing pH value. Solutions adjusted to pH 3.6 and 5.0 with hydrochloric acid gave similar results to those adjusted with sulphuric acid.

Solutions of 0.1 M, 0.03 M, 0.01 M, and 0.001 M concentrations were adjusted to pH 5.0 with sulphuric acid and compared in toxicity with solutions of the base of the same molar concentrations. At each nicotine concentration, the free base is about 5 to 7 times more toxic than is nicotine sulphate solution at pH 5.0.

The addition of an inorganic hydroxide (0.05 to 0.001 N) to an aqueous solution of nicotine is without apparent effect upon the toxicity of that solution.

Pyridine solutions of 0.03 M and 0.12 M were about twice as toxic as solutions of the same molar concentration titrated to pH 5.0 and 4.9, respectively, with sulphuric acid. Methylpyrrolidine solution (0.03 M) was about 19 times more toxic than methylpyrrolidine hydrochloride solution of pH 3.0.

The speed of toxic action to the larva of *Culex pipiens* of nicotine, pyridine, and methylpyrrolidine in aqueous solution is directly related to the concentration of the undissociated molecules.

It is believed that toxicity results largely from the penetration of the molecules into the body through the wall of the alimentary tract. Nicotine ions are somewhat toxic but much less so than nicotine molecules. It is also believed that the change in toxicity of a nicotine solution with change in pH results largely from the dissociation of the pyrrolidine nitrogen.

Previous writers have explained the greater toxicity of nicotine over nicotine sulphate on the basis of the greater volatility of the former. In this study it is shown that the free base in solution is also much more toxic than nicotine sulphate.

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EFFECT OF CERTAIN CLIMATIC FACTORS ON THE DIAMETER GROWTH OF LONGLEAF PINE IN WESTERN FLORIDA¹

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INTRODUCTION

Studies of the properties of wood show fluctuating variations that can be attributed only to differences in the structure of the test pieces. Within any one species of tree such variations result from the influences of environmental, and perhaps inherent, factors; hence the departures in properties of the resultant timbers are explained by the effects of these factors on xylem formation. Two methods of approach to the problem present themselves: (1) A study of growth and structure under such conditions that any one environmental factor may be regulated at will; and (2) a statistical analysis of existing stands to determine through correlation the effect of a given factor on growth. The second method has been followed in this investigation.

LITERATURE REVIEW

Data on the inherent factors that exert an influence on diameter increases in woody plants (movements of food materials and water, enzymatic activity, hereditary cyclic activity, and the like) are so meager that statistical methods can not be employed in their analyses. Of the more potent fluctuating environmental factors, precipitation and air temperature are the only ones on which there are records over extended periods of time. Hence the influence of only these two factors can be considered at present.

Precipitation has long been considered as one of the most important factors exerting a controlling influence on growth. Douglass (6, p. 10)³ concluded as follows:

In a considerable number of cases, but especially in the dry-climate groups [this [correlation between precipitation and diameter growth] has been found to be in the neighborhood of 70 per cent, which is raised substantially by applying a formula to allow for some degree of moisture conservation.

Trees grown in moist climates showed a rhythmic variation in the width of the annual rings of growth that corresponded to the solar activity. The effect of sun spots on tree growth appeared to be through their influence on rainfall. Shreve (17) noted a weak qualitative correlation between diameter growth and annual rainfall in Monterey pine, and a small positive relationship in redwood with the December-September precipitation, indicating the beneficial influence of the late winter rainfall on growth in the succeeding season: Pearson

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³ Reference is made by number (italic) to Literature Cited, p. 362.

(14) decided that the precipitation during April and May had more effect on height growth in western yellow pine than did that during any other portion of the year. Brewster (2) was unable to find a definite relation between height growth in western larch and precipitation during the early growth months (April-July). Burns (3), working in the Vermont forests says, "There can be no direct correlation between rainfall and diameter growth." Conard (4) failed to locate a correlation in Iowa between the rate of growth of oaks and rainfall. Stewart (18) showed that the June-July precipitation was more closely related to diameter growth in oak than was the rainfall for the growing season. He studied the annual rings of growth in but one stump, and used records from a meteorological station 25 miles distant. Bogue (1), working in Michigan, found that an abnormally small or large precipitation was indicated in the ring of the following year, though Robbins (15) could find no evidence of such a delayed effect. Hartig and Weber (8) noted that the annual ring in beech formed during a wet year was only 80 per cent of the width of the previous ring, while spruce in the same locality possessed a normal increment.

Attempts to correlate the progression of xylem formation with temperature have not been so successful as have correlations with rainfall. Schwarz (16) has recorded a definite relationship between the January-March temperature and ring width in *Pinus sylvestris* Linnaeus. The widest rings were formed in the years having a mean monthly temperature above a 22-year average, and with few 5-day periods in which the average temperature was below the freezing point. Conversely, narrow rings were produced whenever the temperature for the first four calendar months was below the average and showed numerous 5-day periods in which the mean fell below 0° C. Nördlinger (13) believed that a warm moist year must favor the development of the ring, and that the preceding winter precipitation played a part. Shreve (17), working with Monterey pine, summed all the temperatures above 40° F. and concluded that there was no correlation between the result and tree growth. He stated that "there is rarely justification for attributing irregularities of growth under natural conditions to the fluctuation of a single environmental condition." Robbins (15) found that in the red oak group ring width varied inversely as the sum of the mean temperatures for May and June, though a direct correlation with the March-June rainfall was also present. Stewart (18) reached no conclusions as to the relation between temperature and growth. Pearson (14) noted that height growth in young western yellow pines varied inversely as the temperature and attributed this to the effect on transpiration and water supply.

EXPERIMENTAL FIELD CONDITIONS

Longleaf pine (*Pinus palustris* Miller) was selected for the Forest Products Laboratory study presented here, partly because of its high rating as a structural timber, and partly because the results might be applicable to other experiments under way. The data were obtained on the Choctawhatchee National Forest in western Florida, approximately 50 miles east of Pensacola and about 7 miles from the Gulf of Mexico. The particular trees examined in this investigation

were growing on Section 15, Range 23 West, Township 1 South (Tallahassee Meridian).

This region receives an annual rainfall of about 60 inches. The mean for the early spring months is lower than that for the others, while July and August show a greater precipitation. Most of the rainfall during the vegetative season occurs in heavy showers over small areas. The growing season, as determined by the first and last frosts, is from early February until late November. The weather records of the Camp Pinchot ranger station, $4\frac{1}{2}$ miles to the southwest of the trees studied, were used throughout this investigation.

The portion of the township in which the study was conducted supports a rather open stand of longleaf pine (*Pinus palustris* Miller) with its attendant understory of turkey oak (*Quercus catesbaei* Michaux), post oak (*Q. stellata* Wangenheim), blue-jack oak (*Q. cinerea* Michaux), and palmetto (*Serenoa serrulata* (Michaux) Hooker). The stand is on a poor site. The trees, which are slow in growth, range from 150 to 250 years of age.

This area has an almost imperceptible slope to the east and northeast, and the subterranean water movements are in the same direction. The soil is Norfolk sand of undetermined depth and with a maximum capillary water-holding capacity of 8 per cent. Some of the larger trees had been cut at various times on this forest area prior to 1908, the stand has not been turpented, and has suffered little from fire since 1914, when it was acquired by the Forest Service. The available records show a possible light fire in 1920, and controlled burning to remove debris in 1924 and in 1926.

Fifty-seven trees, ranging in diameter from 2 to 19 inches at breast height, were selected for study. They were carefully measured and described in the field, and increment cores were taken at a height of $4\frac{1}{2}$ feet above ground from four sides, corresponding to the cardinal compass points. All measurements of ring widths were made under a microscope with the aid of a mechanical stage permitting readings to 0.1 mm. and were made continuously from the outside toward the center. In this way any error in measurement or computation was restricted to the half rings in which it occurred.

RELATION BETWEEN TEMPERATURE AND WIDTH OF RING

The investigations of the effect of temperature on xylem production in longleaf pine in western Florida have led to no positive results. In no case was there a combination of monthly mean temperatures that could be considered as influencing diameter growth. Summations of the temperatures above 40° F. in hour-degree units showed such a close correspondence between the various years that no conclusions could be drawn from them. Shreve (17, p. 115) had found previously that the same conditions held for Monterey pine in the "equable maritime climate of Carmel," Calif.

RELATION BETWEEN PRECIPITATION AND WIDTH OF RING

A review of the literature indicates that in many localities precipitation exerts a measurable influence on ring width in trees. Consequently a relationship between these factors was sought. Preliminary investigations indicated that the xylem of longleaf pine in western Florida is produced from February until November, the exact duration

depending upon the earliness or lateness of the seasons. Following Douglass's method of using rainfall from the end of one vegetative period to the end of the next, the precipitation from November 16 to November 15 was compared with the ring produced during that period. No agreement was found. An analysis of conditions in Florida will show that this negative result might have been expected. All the precipitation in this region occurs as rain, which is not stored in the deep, loose soil. In the vicinity of Flagstaff, Ariz., where Douglass worked, the precipitation during the dormant season occurs as snow, which is largely released as available water at the beginning of vegetative activity and is retained for a prolonged period in the heavier soil. In longleaf pine in western Florida, then, no allowance for appreciable storage of winter precipitation should be made.

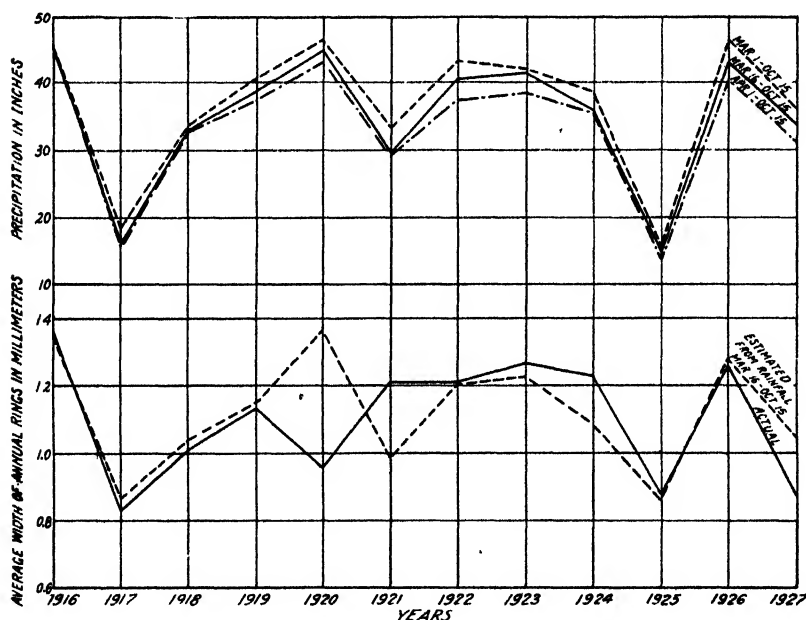


FIGURE 1.—Comparison of seasonal precipitation and average width of annual growth rings by years

Other investigators have indicated the effect on tree growth coming from rainfall that occurs during the early growth months. No such relationship exists in the longleaf pine studied. This may also be attributed to the small storage capacity of the soil and as already stated to the somewhat lower precipitation in this region during the early spring months.

On the other hand, the precipitation occurring during the vegetative season does affect diameter increment, as is evident by reference to Figure 1. The average ring width for each of the 57 trees investigated was derived from four radii. With the exception of the years 1920 and 1921, which are discussed later, the ring width agrees remarkably well with the precipitation. This can be seen best by comparing the curve in Figure 1 showing actual ring width with that show-

ing the width as estimated from the seasonal precipitation. The estimation was based on an average curve in a graph in which ring width was plotted against precipitation during the period of March 16 to October 15 for the years 1916 to 1927, inclusive. The other deviations in the two curves may easily have resulted from varying vegetative periods occasioned by early or late seasons, or from irregular seasonal distribution of the precipitation, although an inspection of the records does not show that the years 1924 and 1927 had exceptionally uneven daily distribution of rain. There is little choice between the period from March 1 to October 15, inclusive, and that beginning 15 or even 30 days later, probably because of the effect of reserve foods, which is discussed later. A qualitative correlation between these values was obtained through the method used by Shreve (17). Each year in which the ring width increased or decreased with the precipitation was considered as a positive agreement, and when the two disagreed in direction it was deemed negative. In this way the correlation is 64 per cent using the March 1 to October 15 rainfall, and 73 per cent using that from March 16 to October 15 as well as that from April 1 to October 15. Excluding the years 1920 and 1921, the correlations are 78, 89, and 89 per cent. Two somewhat longer periods, namely, February 16 to October 15, and March 1 to October 31, were discarded because an abundance of rainfall in late February and late October of certain years did not appear to have a corresponding effect on xylem formation. The foregoing data indicate that during the first few weeks of cambial activity sufficient soil moisture, or stored water within the plant, is present under the conditions of relatively low transpiration to allow growth to progress at approximately its maximum rate, and that during the last few weeks growth is so sluggish because of inherent or external factors that it can not be stimulated by an additional supply of water.

The total disagreement of precipitation and xylem production for 1920 and 1921 (fig. 1), regardless of the period for which the precipitation was computed, is not fully explained. Records of the Forest Service show, however, an especially heavy seed crop for longleaf pine in 1920. If this phenomenon were to explain the inconsistencies observed in the curves, there should be some relation between the decrease in ring width and the number of cones borne. The Southern Forest Experiment Station of the United States Forest Service has kindly furnished data as to the number of cones produced on trees of different diameters during a partial seed crop in 1924. Few cones were found on trees up to 8 inches in diameter. The number per tree increased from there on up to a tree diameter of 14 inches, and then decreased. On the basis of this information the trees studied were grouped into diameter classes, and the average ring widths for each group are presented in Figure 2. A comparison of these graphs shows that decreases in radial increments during 1920 as compared with 1919 are roughly in proportion to the potential seed-producing capacity of the various groups.⁴ When compared to the expected growth from the precipitation in these two years other facts are evident. The rainfall in 1920 was above that for 1919, hence a wider

⁴ It should be observed that while from 1919 to 1920 the growth fell off about the same amount in the trees 5 to 8 inches in diameter as in those 9 to 12 inches in diameter, the decrease on a percentage basis was less in the smaller trees because in general they had wider rings.

ring would be expected. (Fig. 1.) This, coupled with the decrease that actually occurred, accentuates the apparent effect of seed production on ring width.

On the other hand, though the 1921 ring would be expected to be relatively narrow because of moderate precipitation in that year, the rings were actually wider than would be expected from the rainfall, as is indicated in Figure 1. This increase in width of rings may be due to a larger amount of food material stored in the tree or to a stimulus of some kind carried over from the preceding year. Since the seed production did not decrease the width of rings for the following year, two possibilities as to the source of the food material necessary for seed maturation present themselves. Either the food reserves are utilized during the seed year at the expense of ring width, and new reserves are built up later in the season to be used during the following

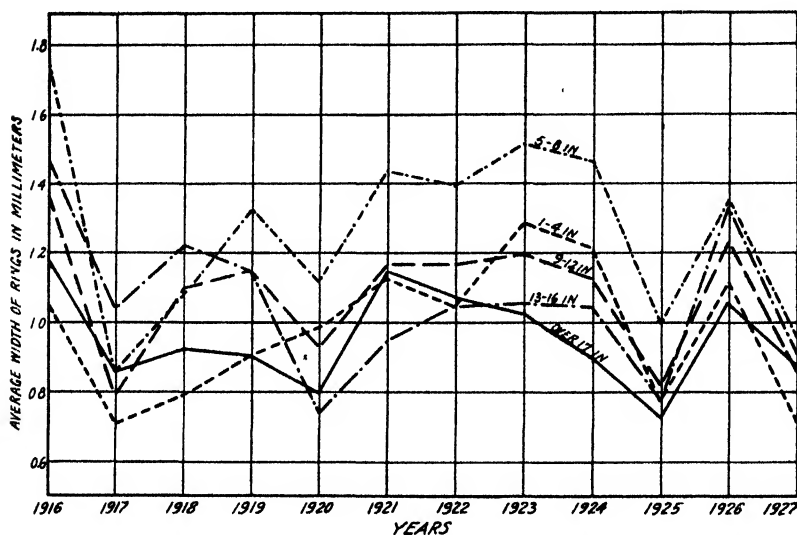


FIGURE 2.—Comparison of average width of annual growth rings in trees of different diameter classes

year, or the reserves of the year preceding fruit production are held in abeyance to be used in xylem production the year after fruiting, and the food materials produced in the current year are diverted largely to seed formation.

Whether the seed year of 1920 actually was the cause of the inconsistencies of ring width in comparison to rainfall in 1920 and 1921 can not definitely be determined, but at present it is the only plausible explanation. Subsequent seed crops, especially in years of abundant rainfall, will be necessary for more conclusive evidence.

EFFECT OF VIGOR OF TREES IN RESPONSE TO RAINFALL

The number of individuals necessary to secure reliable results in any problem to which statistical methods may be applied is always a matter of interest. Douglass (6) found that 5 trees of western yellow pine gave almost the same growth, even to small details, as did

15, but that there was less agreement between the oldest pair than among the other three. It should be recalled that most of his trees were of great age. The fact that the older trees, which were probably less vigorous, varied from the mean is also significant.

The preceding discussion has made evident the fact that trees of different diameters vary in response to stimulation. It remains to be

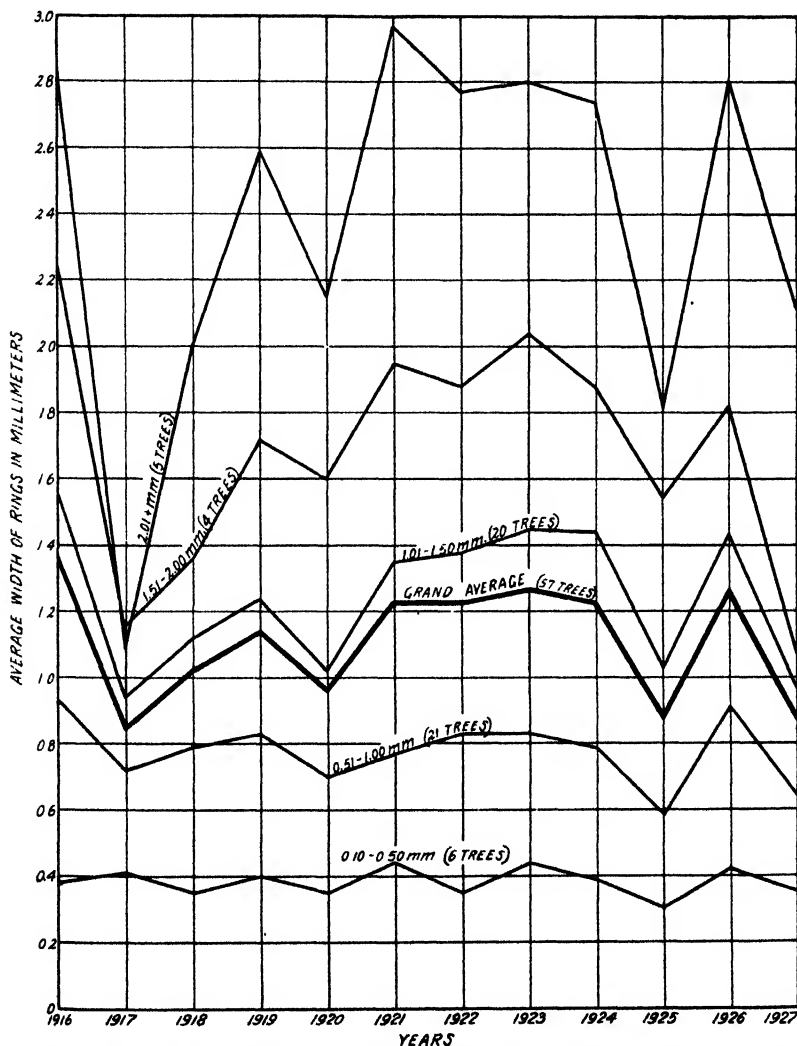


FIGURE 3.—Comparison of average width of annual growth rings by vigor classes as determined by average width of rings

determined whether trees of the different vigor classes show comparable growth responses and whether the results secured from one side of the tree are comparable to those secured from four radii. Investigations of these points were made by comparing curves, and with no attempt to develop numerical comparisons.

Vigor classes were determined from the average width of rings grouped by $\frac{1}{2}$ -mm. intervals for 12 years (1916–1927). The mean of each of these arbitrarily selected vigor classes gave a curve that follows with remarkable similarity the general curve for the 57 trees. (Fig. 3.) The departures usually are in amount rather than in direction. The groups with a growth approaching the average show an almost exact duplication of the general curve; the group of slowest growth shows the least similarity in direction and amount of growth. Except for this group of slowest growth, the responses to rainfall were so similar that on a percentage basis they were practically identical in trees of various rates of growth. It is also obvious from Figure 3 that vigorous trees are better visual indicators of the abnormal years than are trees of slow growth, and that narrow rings serve best as indicators to be used in the cross-identification of rings in different trees. Douglass (6) also reached similar conclusions.

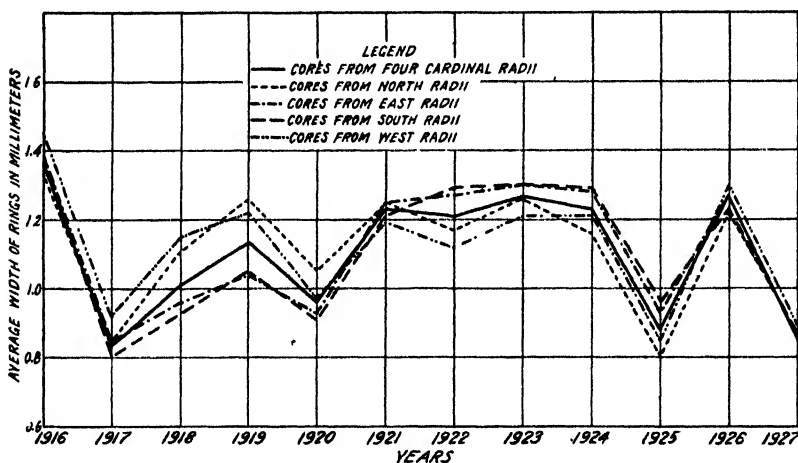


FIGURE 4.—Comparison of average width of growth rings along four individual cardinal radii with the average of four

RELIABILITY OF ONE CORE AS COMPARED TO FOUR

The average growth curve obtained from four cores taken at the cardinal compass points on each of the 57 trees is compared to the average curve for each of the radii in Figure 4. A number of departures of the separate radii from the average for all four may be noted, especially in certain years, although in general the data obtained from any one radius are of about the same magnitude of reliability as the data secured from averages of four cores on different sides when taken from a considerable number of trees.

CARDINAL DIRECTIONS SHOWING GREATEST GROWTH

It is occasionally stated that in north temperate regions straight trees growing on an essentially level site will show an eccentricity in diameter, and that the south and west sides will evince the greater growth. This is attributed to a longer period of activity on these exposures because of greater insolation. Lodewick (10) was unable to find evidences of longer cambial activity on the south sides of trees

investigated by him, while Douglass (6) reported greatest growth on the northeast side in western yellow pine because the slope of the area on which he worked made soil water first available on that side. The water factor would hardly be operative in the longleaf pine of western Florida where an excess of water in the soil passes downward so readily that lateral movements undoubtedly occur only below the reach of all except the taproot. If such a factor were operative the south and perhaps the west radii should be the longer owing to the coordination of this factor and insolation. The data presented in Figure 4 do not indicate consistently greater increments along any one radius. The cores from each tree were studied separately, and the number of times the total growth for the last decade on each radius was greatest, next greatest, and so on, was recorded. The results for 48 trees, which are given in Table 1, show that the growth along the north and the west radii was larger than along the other two radii. This might presuppose a northwest-southeast eccentricity, though an examination of 12 trees that had recently been cut along a right of way gave no evidence of consistent elongation of any one axis. The decadal growth on each radius permits a similar interpretation in that the north and west sides show a greater total growth.

TABLE 1.—*Relation between cardinal directions and diameter development at breast height in 48 longleaf pines in western Florida*

Radius	Number of times length of each radius ranked—				Average width in mm. of 10-years' growth (1918-1927) on each radius
	First	Second	Third	Fourth	
North.	18	13	10	7	12.0
East.	10	9	18	11	11.4
South.	4	14	12	18	11.2
West.	16	12	8	12	11.9

DIAMETER ECCENTRICITY AS RELATED TO CROWN DEVELOPMENT

It is generally supposed that with a restricted horizontal movement of food materials in a tree the diameter increments of that portion of the xylem connected with the larger branches or the greatest number of branches should be the greatest (11). Whether or not exposure at breast height directly under the greatest photosynthetic area will show these xylem increments depends upon the straightness of the grain through the intervening bole. Spiral grain, as seen in dead and peeled trees, is frequent in longleaf pine on the Choctawhatchee Forest, though no bark or other external criteria have been found to indicate spiral grain in trees with the bark on. Consequently no information on spiral grain in the trees studied was obtained. The data on the relation between crown development and eccentricity in the bole at breast height have been summarized in Table 2.

TABLE 2.—*Relation between crown and bole development in longleaf pines in western Florida*

Crown development	Number of trees	Radius examined	Bole development—Number of cardinal radii showing growth for 10 years (1918-1927), that is—		
			Least	Medium	Greatest
North least	6	N.	2	0	2
		E.	3	2	0
		S.	1	2	0
		W.	0	2	4
East least	6	N.	0	1	2
		E.	1	0	3
		S.	2	4	0
		W.	3	1	1
South least	3	N.	1	0	1
		E.	2	0	0
		S.	0	2	1
		W.	0	1	1
West least	6	N.	2	0	2
		E.	3	2	0
		S.	1	2	0
		W.	0	2	4
Symmetrical	17	N.	2	4	7
		E.	5	5	3
		S.	4	6	1
		W.	6	2	6

In the trees studied there is no consistent decrease in diameter on the side of least crown development when measurements are made at breast height; in fact, according to the data in Table 2, an increase in diameter was more often the case. In trees supporting a symmetrical crown the north and west sides show a better growth, agreeing with the results obtained when all trees were studied regardless of crown development. The data permit no conjectures as to the reason for this.

CROWN SIZE AND RING WIDTH

An attempt to correlate crown size with diameter increments met with failure. The solid shape of the crown in longleaf pine varies from oval in young trees through a truncated cone to nearly a disk in old trees. By using the cone as the standard, the crown volume of each tree was calculated and compared with the average diameter during the last decade. So little agreement was found, even among similar crown shapes, that it was deemed unnecessary to make more accurate volume computations.

FACTORS AFFECTING SUMMER WOOD PRODUCTION

It has already been shown that the ring width during any one season evinces a close agreement with the precipitation in that period. As a result, it is logical to expect that the rainfall during the time of summer wood formation also affects that portion of the ring. Investigations of longleaf pine during 1927 showed that the heavy-walled part of the ring started during late May or June, the exact time depending upon the season, and continued until the cessation of growth in November. Trials with various beginning and ending

dates on longleaf pine have led to the conclusions that the precipitation from about the middle of June to the middle October has the greatest effect on the production of summer wood, the qualitative correlation in this case being 91 per cent. The curves presented in Figure 5 show that, except for one or two years, there is little choice between the seasons beginning June 1, June 16, or July 1. The disagreement that was noticeable in the total ring width in 1921 is also evident here, but in 1920 the summer wood formation fell off with the rainfall.

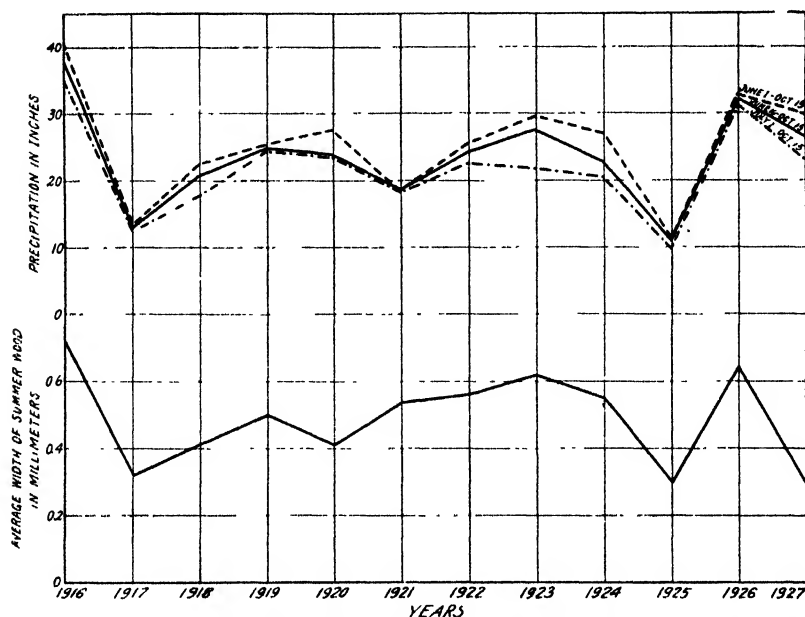


FIGURE 5—Comparison of seasonal precipitation and average width of summer wood by years

RELIABILITY OF SUMMER WOOD DETERMINATIONS FROM ONE SIDE OF TREE

The reliability of the percentage of summer wood obtained by measurements on one radius as compared with the results from four radii on the same tree was determined by computing the mean and probable error in each measurement. Twenty-five trees were used with the following results:

	Mean summer wood per cent
Four radii.....	43.30 ± 0.246
North radius.....	44.07 ± .490
East radius.....	43.07 ± .499
South radius.....	43.21 ± .480
West radius.....	42.85 ± .485

It is evident that the use of one radius from 25 trees gives results sufficiently accurate for most purposes.

FACTORS AFFECTING SPRING WOOD PRODUCTION

The foregoing discussion has shown that the summer wood is affected by the precipitation of the vegetative period beginning between June 1 and July 1, and, therefore, it is logical to expect that the rainfall during the early portion of the growing season affects the production of spring wood. Figure 6 presents the average spring

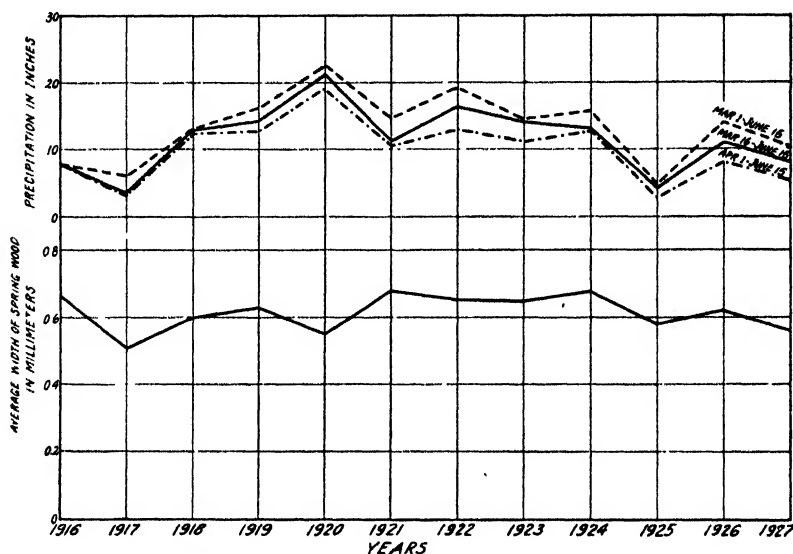


FIGURE 6.—Comparison of seasonal precipitation and average width of spring wood by years

wood width for the 57 trees and the precipitation for three periods during the early months. All three of the periods give fair agreement with spring wood production, though the spring wood did not fluctuate with rainfall to so great a degree as did the summer wood. (Figs. 5 and 6.) The departures are mainly quantitative, the qualitative ones being confined to the years 1920, 1921, 1922, and 1923 for each of the periods, and also for 1924 for the period March 16 to June 15. Apparently other factors also exert an influence on spring wood production.

Since the time of Hartig there has been an hypothesis that the spring wood portion of the ring is formed largely at the expense of stored food reserves (9). This seems true enough in the deciduous ring-porous species which, as Lodewick (10) has shown, complete the production of the early part of the ring prior to final leaf expansion. There may be some doubt, however, for evergreen species where the old foliage is capable of photosynthetic activity during the period of spring wood formation because the newly elaborated food might be directed to wood production, to foliage development, or to both. De Smidt (5) and Myer (12) have shown that the ring width is proportional to the volume available for food storage. If the spring wood of any one year is partially dependent upon the stored reserves, a certain amount of spring wood should be formed as long as the rainfall during the early months is sufficient to release the stored reserves

for growth. Figure 7 shows that as the precipitation increases the yearly growth of spring wood formed per unit of water decreases;⁵ in fact, except for slight variations, as is also borne out by Figure 6, the spring wood formation is almost a constant, irrespective of rainfall. The summer wood, on the other hand, appears to vary more directly with the rainfall, as is indicated by its more nearly horizontal direction in Figure 7. A combination of the two curves would give an intermediate result for the variation of the total ring width.

Shreve (17) utilized similarly derived values in his measurement of the relationship between precipitation and growth, and concluded that there was no relationship between the two because slow-growing trees possessed a higher growth rate per unit of precipitation. An analysis similar to the foregoing would undoubtedly have shown that allowances for the wood formed by reserves should have been made in western yellow pine as well as in longleaf pine.

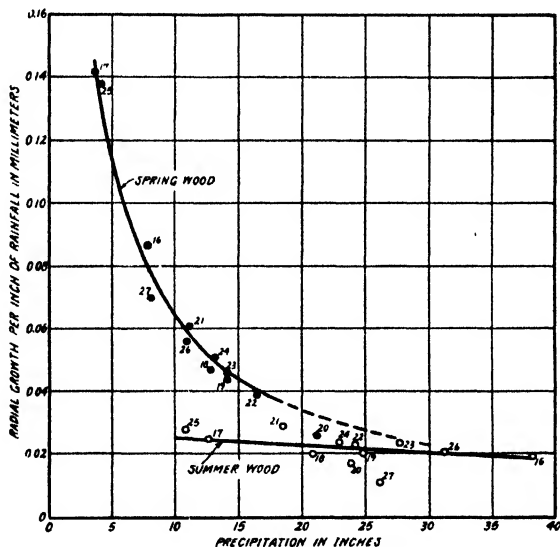


FIGURE 7.—Relation of seasonal growth to seasonal precipitation. The numbers correspond to the years for which the measurements were made

SUMMARY

In an investigation of the relation between certain climatic factors and diameter growth in longleaf pine on the Choctawhatchee National Forest in western Florida no effect of temperature on wood production was found. There was a fairly definite relation between the width of the annual rings and the precipitation from March 16 to October 15 except for the years 1920 and 1921, for which the seed crop of 1920 and its reaction during the following year may have been responsible.

Marked reduction or increase in precipitation was accompanied in most cases by corresponding variations in the ring width.

⁵ In Figure 7 the lower extension of the spring wood curve is based on an apparent maximum yearly production of 0.7 mm. spring wood obtained under a water supply equivalent to fully 30 inches of rainfall during spring on an irrigated plot in western Florida.

Vigorous trees were better visual indicators of the effect of climatic factors on wood production than were nonvigorous trees, since the sinuosities of their growth curves were more marked.

Dry years and their accompanying narrow rings were found to be the most reliable for use in the cross identification of rings in different trees.

The data from one radius on 57 trees was about as reliable as the data from four radii on the same trees.

No marked relationship between cardinal directions and diameter growth at breast height was found in the trees investigated.

No relationship between unsymmetrical crown development and eccentric diameter growth was found in the trees investigated.

There appeared to be no correlation between crown size and rate of diameter growth.

The precipitation during the latter part of the growing season (from June 1 to July 1 until October 15) exerted the principal controlling influence on summer wood formation.

The percentage of summer wood as determined from one radius on 25 trees was about as reliable as that from four radii on the same trees.

The precipitation accompanying the period of spring wood formation showed a fair qualitative correlation with the width of the spring wood. Quantitatively this correlation was poorer than that for the summer wood; in fact, over a period of years the width of spring wood was almost constant irrespective of rainfall.

The amount of spring wood formed per unit of rainfall decreased as the precipitation for the season increased, but the amount of summer wood formed was almost directly in proportion to rainfall.

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PARASITES OF THE ORIENTAL PEACH MOTH, *LASPEYRESIA MOLESTA* BUSCK, IN NORTH AMERICA¹

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INTRODUCTION

Because of the continued failure to discover a satisfactory control measure for the oriental peach moth (*Laspeyresia molesta* Busck), a pest which is spreading rapidly each year in the more eastern peach-producing areas of the United States and Canada, considerable attention is now being devoted to the insect parasites which attack it.

Complete records of the presence and effectiveness of parasites of this insect have been published thus far for only a few localities. In view of the fact that a search is soon to be made for foreign parasites which might be introduced into this country to aid in combating this insect, it seems advisable at this time to prepare a list of those species which have been recorded to date as parasitic on the pest in North America.

Before parasites from other sources are brought into this country a thorough study should be made throughout the entire range of the host to determine what parasites are already present in each locality, and their abundance and effectiveness. The writer has conducted such a study at Riverton, N. J., for the peach district represented by that locality. The information obtained regarding the parasites in that district is presented here in detail, with a description of some of the methods which proved to be most satisfactory for conducting a survey of oriental peach moth parasites in any locality.

INSECTS RECORDED AS PARASITIC ON THE ORIENTAL PEACH MOTH IN NORTH AMERICA

In connection with biological studies on the oriental peach moth conducted at Riverton and Moorestown, N. J., during four seasons (1925 to 1928, inclusive) the writer found 28 species of primary parasites attacking this host, and 5 species of secondary parasites. By supplementing these records with those obtained from published data and through correspondence,² the list of insects recorded as parasitic on the oriental peach moth and presented in Table 1 has been prepared.³ Information regarding the locality from which each

¹ Received for publication Apr. 2, 1930; issued September, 1930.

² In February, 1927, a survey of the more important parasites and predators of the oriental peach moth was compiled by Alvah Peterson and the writer from reports submitted by several cooperators through correspondence and from published data. For the records taken from that survey, from published data, and from correspondence, the writer is indebted to the following workers (who furnished data from the Province and States named in parentheses): W. A. Ross (Ontario, Canada); Philip Garman (Connecticut); D. M. Daniel (New York); L. A. Stearns, B. F. Driggers, and Alvah Peterson (New Jersey); A. B. Champlain, J. R. Stear, S. W. Frost, and J. R. Eyer (Pennsylvania); Philip Garman, H. S. McConnell, and L. A. Stearns (Maryland); L. A. Stearns, W. B. Wood, and E. R. Selkregg (Virginia), and O. L. Snapp (Georgia).

³ The writer is also indebted to R. A. Cushman and A. B. Gahan, of the Division of Taxonomy and Interrelations of Insects, Bureau of Entomology, and J. M. Aldrich, of the Division of Insects, U. S. National Museum, for the determination of specimens, and particularly to Mr. Cushman for checking the list of species in Table 1.

species has been recorded, the stage of the host which is attacked, and the relation of the parasite to the host is included. The list contains 57 species of primary parasites, 5 of which are questionable, and 8 species of secondary parasites. Ten families of Hymenoptera are represented, including 33 species of Ichneumonidae, 16 of Braconidae, 3 of Eupelmidae, 2 of Pteromalidae, and 1 each of Bethyidae, Eulophidae, Eurytomidae, Perilampidae, Tetrastichidae, and Trichogrammatidae. Five species of the dipterous family Tachinidae are also included.

TABLE 1.—List of insects recorded as parasitic on the oriental peach moth in North America

PRIMARY PARASITES

Species	Order	Family	Stage of host attacked	Relation to host	Recorded from—
<i>Actia pilipennis</i> Fallen *	Diptera	Tachinidae	Larva	Internal	New Jersey.
<i>Aenoplex betulacola</i> Ashm *	Hymenoptera	Ichneumonidae	Prepupa ^b	do	Ontario, New Jersey, and Pennsylvania
<i>Aenoplex carpocapsae</i> Cush	do	do	do ^b	do	Pennsylvania
<i>Allocoila thyridopterygis</i> Riley *	do	do	Larva ^b	do	Do.
<i>Angitia</i> sp *	do	do	do	do	New Jersey
<i>Apanteles harti</i> Vier *	do	Braconidae	do	do	Do
<i>Apanteles</i> sp *	do	do	do	do	New Jersey, Virginia, and Georgia
<i>Ascogaster carpocapsae</i> (Vier) *	do	do	Egg	do	Ontario, New Jersey, Pennsylvania, Maryland, and Virginia
<i>Bassus carpocapsae</i> Cush *	do	do	Larva	do	New Jersey
<i>Calliephialtes grapholithae</i> (Cress) *	do	Ichneumonidae	Cocoon ^d	External	New Jersey and Pennsylvania
<i>Calliephialtes</i> n. sp. *	do	do	do	do	New Jersey
<i>Centeterus ineptifrons</i> Gahan *	do	do	do	Internal	New Jersey, Pennsylvania, and Virginia
<i>Cremastus forbesii</i> Weed *	do	do	Larva	do	New Jersey
<i>Cremastus minor</i> Cush *	do	do	do	do	Ontario and New Jersey
<i>Cremastus</i> sp.	do	do	do	do	New Jersey
<i>Cryptus vinctus</i> (Say)	do	do	Cocoon ^b	do	Do
<i>Diapetimorpha orba</i> (Say) *	do	do	Larva ^b	do	Virginia
<i>Diocetes obliteratus</i> (Cress) *	do	do	do	do	Ontario and New Jersey
<i>Encyrtaspis semirufus</i> Gahan	do	Eupelmidae	do	do	Georgia.
<i>Ephialtes aequalis</i> (Prov.) *	do	Ichneumonidae	Cocoon ^b	do	Ontario, New Jersey, and Virginia.
<i>Epiurus indagator</i> (Cress) *	do	do	Larva or prepupa ^b	External	New Jersey and Maryland.
<i>Epiurus</i> n. sp.	do	do	do ^b	do	Ontario.
<i>Eubadizon gracilis</i> Prov. ^f	do	Braconidae	Larva	Internal	New Jersey and Virginia
<i>Eubadizon pleuralis</i> Cress. ^f	do	do	do	do	Ontario and New Jersey.
<i>Eubadizon</i> sp. * ^f	do	do	do	do	Ontario, Connecticut, New Jersey, and Georgia.
A species of the family Eupelmidae.	do	Eupelmidae	do	External	Maryland.
<i>Glypta phoxopteridis</i> Weed *	do	Ichneumonidae	do	Internal	New Jersey.
<i>Glypta rufescutellaris</i> Cress *	do	do	do	do	Ontario, Connecticut, New Jersey, Pennsylvania, and Maryland.

* Bred from *L. molesta* by the writer

^b The stage of host attacked is not known, but the probable stage is given.

^c This species is usually bred from bagworms and tussock moths. Cushman is inclined to doubt that it is parasitic on *L. molesta*.

^d By cocoon is meant either prepupa or pupa within the cocoon.

^e Normally parasitic on spider eggs. Cushman doubts the record.

^f Cushman and Gahan are of the opinion that all these are probably the same and are neither *gracilis* nor *pleuralis* but represent undescribed species.

TABLE 1.—List of insects recorded as parasitic on the oriental peach moth in North America—Continued

PRIMARY PARASITES

Species	Order	Family	Stage of host attacked	Relation to host	Recorded from—
<i>Glypta varipes</i> Cress	Hymenoptera	Ichneumonidae	Larva	Internal	Ontario
<i>Glypta vulgaris</i> Cress		do	do	do	Virginia
<i>Goniozus</i> sp.		Bethylidae	do	External	Do
<i>Hoplocryptus incertus</i> Cush ^a (= <i>incertus</i> Prov.)		Ichneumonidae	Cocoon ^b	Internal	New Jersey.
<i>Hoplectis conquistator</i> (Say) ^c		do	do	do	New Jersey, Pennsylvania, and Virginia
<i>Leskiomima tenera</i> Wied	Diptera	Tachinidae	Larva	do	Maryland.
<i>Leucodesmus nigriventris</i> Gir	Hymenoptera	Eulophidae	do	do	Virginia
<i>Lixophaga plumbea</i> Aldr	Diptera	Tachinidae	do	do	Do
<i>Lixophaga variabilis</i> Coq. ^a	do	do	do	do	New Jersey, Maryland, Virginia, and Georgia
<i>Macrocentrus ancyliivora</i> Roh. ^a	Hymenoptera	Braconidae	do	do	Connecticut, New Jersey, Pennsylvania, Maryland, and Virginia
<i>Macrocentrus delicatus</i> Cress. ^a	do	do	do	do	New York and New Jersey
<i>Macrocentrus</i> sp.	do	do	do	do	Ontario, New Jersey, and Virginia
<i>Meteorus hyphantriae</i> (Riley)	do	do	do	do	New Jersey
<i>Microbracon elegans</i> (Ashm.)	do	do	do	External	New Jersey, Maryland, and Virginia
<i>Microbracon helictor</i> (Say) ^a	do	do	do	do	New Jersey.
<i>Microbracon mellitor</i> (Say)	do	do	do	do	Ontario
<i>Microcryptus</i> sp.	do	Ichneumonidae	Cocoon ^b	Internal	New Jersey
<i>Nemorilla phycitae</i> LeB.	Diptera	Tachinidae	Larva	do	Maryland
<i>Phanerotoma tibialis</i> Hald. ^a	Hymenoptera	Braconidae	Egg	do	New Jersey.
<i>Phygadeuon</i> sp.	do	Ichneumonidae	Larva	do	Do
<i>Pristomerus ocellatus</i> Cush. ^a	do	do	do	do	Do.
<i>Rogus platypterygis</i> Ashm.	do	Braconidae	do	do	Virginia
<i>Sagaritis consimilis</i> (Ashm.)	do	Ichneumonidae	do	do	New Jersey.
<i>Sagaritis patanketorum</i> Vier.	do	do	do	do	Do.
<i>Spilocryptus</i> sp.	do	do	Cocoon	do	Maryland and Virginia
<i>Stilbopoides sesivora</i> Rolt	do	do	Larva	do	New Jersey.
<i>Syntomosphyrum esuius</i> Riley	do	Tetrastichidae	Pupa	do	Do
<i>Trichogramma minutum</i> Riley. ^a	do	Trichogrammatidae	Egg	do	Connecticut, New York, New Jersey, Pennsylvania, and Maryland.
<i>Trichstus curvator</i> (Fab.) ^a	do	Ichneumonidae	Pupa	do	New Jersey and Maryland.

SECONDARY PARASITES

Species	Order	Family	Recorded from—
<i>Cerambycobius</i> sp. (probably <i>Eupelmus amicus</i> Gir.)	Hymenoptera	Eupelmidae	Virginia.
<i>Dibrachys boucheanus</i> (Ratz.) ^a	do	Pteromalidae	Ontario, New Jersey, Pennsylvania, Maryland, and Virginia.
<i>Dibrachys</i> (<i>Tritneptis</i>) <i>hemerocampae</i> Gir. ^a	do	do	New Jersey
<i>Eurytoma</i> sp.	do	Eurytomidae	Maryland.
<i>Gelis</i> sp.	do	Ichneumonidae	New Jersey.
<i>Hemiteles tenellus</i> (Say) ^a	do	do	Do.
<i>Hemiteles</i> sp.	do	do	Do.
<i>Perilampus</i> sp. ^a	do	Perilampidae	Do.

^a Bred from *L. molesta* by the writer.^b The stage of host attacked is not known, but the probable stage is given.^c Normally a parasite of meal moths.

Although the oriental peach moth has so many insect parasites, very few seem to be of much importance. Those of chief importance are *Macrocentrus ancyliivora* (fig. 1) and *Glypta rufiscutellaris* (fig. 2), both of which attack the larval stage, and *Trichogramma minutum*, which attacks the egg.

Macrocentrus ancyliivora particularly, and *Glypta rufiscutellaris* to a certain extent, are aiding considerably in reducing the annual infestation in

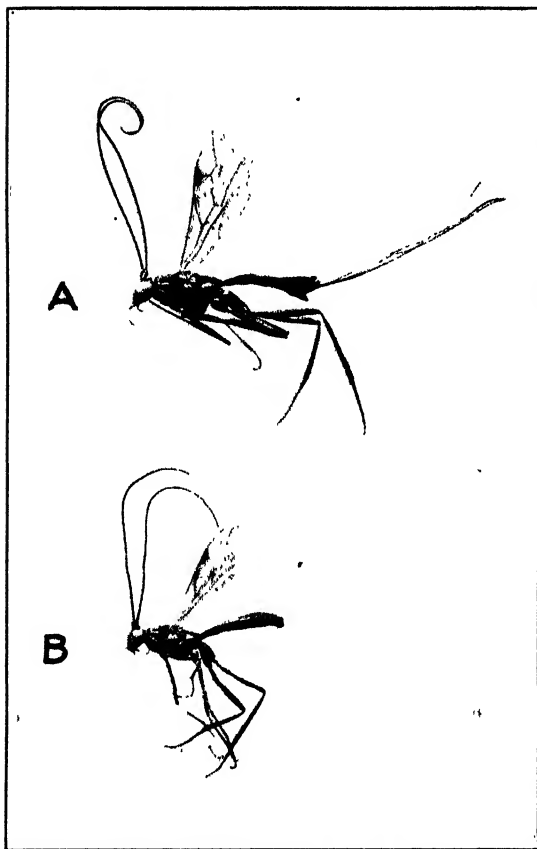


FIGURE 1.—Adults of *Macrocentrus ancyliivora*: A, Female, B, male.
X 5

those sections of New Jersey where they have become established. The work of these two species is confined largely to the earlier broods of host larvae that feed in peach twigs. By destroying large numbers of larvae of the first and second broods early in the season they bring about a considerable reduction in the number of individuals produced in the later broods, most of which feed in the fruit. As pointed out by Stearns,⁴ these two species are apparently limited in their distribution by environmental factors. Thus far, both species have not seemed able to become successfully established and effective in the same locality.

The egg parasite *Trichogramma minutum* has been found to attack large numbers of oriental peach-moth eggs placed in peach orchards at Riverton,

N. J. Garman⁵ and McConnell⁶ both record this species as attacking eggs of the oriental peach moth in Maryland. Garman also records it from Connecticut,⁷ and Stear records it from Pennsylvania.⁸

Of the remaining species, only a few have been recorded as fairly abundant in some localities. The majority are merely occasional records, usually of only a few individuals.

⁴ STEARNS, L. A. THE LARVAL PARASITES OF THE ORIENTAL PEACH MOTH (*LASPEYRESIA MOLESTA* BUSCK) WITH SPECIAL REFERENCE TO THE BIOLOGY OF *MACROCENTRUS ANCYLIIVORA* ROHWER. N. J. Agr. Expt. Sta. Bul. 490, 24 p., illus. 1928.

⁵ GARMAN, P. THE ORIENTAL PEACH PEST (*LASPEYRESIA MOLESTA* BUSCK), A DANGEROUS NEW FRUIT INSECT OF MARYLAND. Md. Agr. Expt. Sta. Bul. 209, 16 p., illus. 1917.

⁶ MCCONNELL, H. S. THE ORIENTAL FRUIT MOTH. Md. Agr. Expt. Sta. Bul. 298: 179-180. 1928.

⁷ GARMAN, P. WORK WITH THE ORIENTAL PEACH MOTH IN 1926. Conn. Agr. Expt. Sta. Bul. 285: 234-239. 1927.

⁸ STEAR, J. R. THE ORIENTAL FRUIT MOTH IN PENNSYLVANIA. Penn. Dept. Agr. Bul. v. 12, no. 8, Gen. Bul. 477, 13 p., illus. 1929.

PARASITISM OF THE ORIENTAL PEACH MOTH IN THE VICINITY OF RIVERTON, N. J.

METHODS OF OBTAINING RECORDS

The presence of parasites which attack and develop within the egg of the host was determined by placing oriental peach-moth eggs, which had been deposited on the leaves of peach or pear twigs in the insectary, in peach orchards. The twigs, in small bottles of water, were placed on posts in such a position as to be in the foliage of the peach trees. After being exposed for 24 hours or longer, the eggs were taken to the insectary and examined to see if they were parasitized.

Most of the information regarding the species of parasites, which attack or develop within the larva of the oriental peach moth previous to the time the cocoon is spun, was obtained by collecting peach twigs infested with the larvae. Collections were made from several orchards during the four consecutive seasons from 1925 to 1928, inclusive, usually once a week in each orchard from the time the first infested twig was found in the spring until twigs containing larvae could no longer be found in early fall. The collecting was usually continued for a definite number of minutes in each orchard, and the twigs were

taken to the insectary immediately. During the first three seasons all the larvae were removed from the twigs, sorted into various size

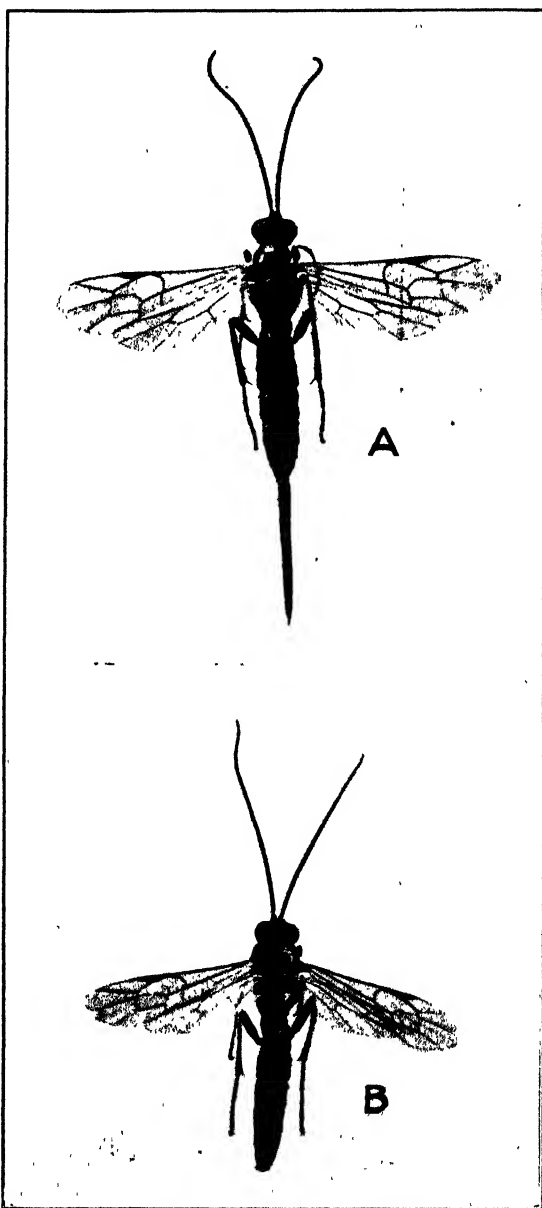


FIGURE 2.—Adults of *Glypta rufiscutellaris*: A, Female, B, male. $\times 4$

according to their length in millimeters, and placed on fruit (peaches or apples) in jelly glasses covered with cheesecloth held in place by rubber bands. They continued their development in the fruit, and then spun cocoons in strips of corrugated straw paper, which were placed in the glasses. The cocoons were removed daily and placed in individual homeopathic vials in which the adult moths or parasites later emerged.

It was found that in rearing the larvae in the insectary after having removed them from the twigs a high mortality occurred, especially in the case of young larvae. Many larvae were injured while being removed from the twigs and died before they could eat their way into the fruit upon which they were placed. Preliminary trials in 1927 indicated that lower mortality resulted if the larvae were not removed from the twigs but were allowed to crawl out at will and go to the fruit. Consequently, throughout 1928 the larvae were removed from only one-half of the twigs taken at each collection. The leaves were trimmed from the remaining twigs, which were shortened to the 3 or 4 inches that contained the larvae. These stems were then placed in 6 by 8 inch glass jars about half full of apples. The more mature larvae, which were able to complete their development within the small portion of twig, crawled out when full grown and spun cocoons in strips of corrugated paper placed in the jars. Larvae which were unable to complete their growth before the twigs dried out abandoned the twigs and completed their feeding in the apples. These also eventually spun cocoons in the corrugated-paper strips. The cocoons were removed about once a week and placed in individual vials until the moths or parasites emerged. Although the same number of infested twigs was handled by each method throughout the entire season, only 1,030 adults (moths and parasites) emerged from the larvae which were removed from the twigs, as compared with 1,433 adults which emerged from the larvae that were allowed to come out of the twigs at will. In other words, 39.1 per cent more individuals completed their development successfully when the larvae were handled by the second method. By being able to rear a larger number of larvae to maturity, a more accurate record can be obtained of the percentage of parasitism occurring in the field. In addition, considerable time is saved because it is not necessary to remove larvae from the twigs.

Additional records of parasites of the larvae were obtained by collecting infested peach and quince fruit in the summer and fall and rearing the larvae to maturity.

Records of the parasites which attack the oriental peach moth while it is a prepupa or a pupa within the cocoon were obtained by collecting cocoons that were spun under the bark of peach and quince trees and rearing the insects to maturity in the insectary. Occasionally larval parasites also emerged from these cocoons, when the host larva had been parasitized before the cocoon was formed. On several occasions strips of corrugated paper containing prepupae or pupae in cocoons were tacked to the trunks or branches of peach trees for several days, but no parasitism occurred.

PARASITISM OF EGGS

Trichogramma minutum was the only parasite found that attacks and develops entirely within the egg of the host. This parasite was present at various times throughout each growing season, and on some occasions 50 per cent or more of the host eggs that were placed on posts in peach orchards were parasitized. Information is not yet available concerning the percentage of parasitism by this species in eggs deposited normally by the oriental peach moth in the orchards near Riverton.

Two species of parasites, *Ascogaster carpocapsae* and *Phanerotoma tibialis*, oviposit in the egg of the host but complete their development within the larva. These two species are treated as larval parasites.

PARASITISM OF LARVAE FEEDING IN PEACH TWIGS

RELATIVE ABUNDANCE AND IMPORTANCE OF THE VARIOUS SPECIES

In the four orchards from which twigs were collected during the seasons of 1925 to 1928, inclusive, 14 species of parasites were found which attack or develop within the larva of the oriental peach moth while the larva is feeding in peach twigs. Table 2 shows the species present each season and the relative importance of each. For the four seasons, 91 per cent of all the larval parasites present were of the species *Macrocentrus ancylicivora*. *Cremastus minor* accounted for about 3 of the remaining 9 per cent, *M. delicatus* for between 2 and 3 per cent, *C. forbesii* and *Glypta rufiscutellaris* for about 1 per cent each, and all the other species together for only about 1 per cent. *Dibra-chys* (*Tritneptis*) *hemerocampae* is a secondary parasite bred from a cocoon of *M. ancylicivora*. *Perilampus* sp. also is a secondary parasite. The table shows the percentage of oriental peach-moth larvae that were parasitized by each species during each season and for the four seasons combined. Parasitism by *M. ancylicivora* alone ranged from 41.6 per cent in 1928 to as high as 55.3 per cent in 1926, with an average of 48.23 per cent for the four seasons. The percentage of parasitism by no other single species exceeded 2.69 per cent in any given season, and the highest average parasitism over the 4-year period by any species other than *M. ancylicivora* was 1.65 per cent by *C. minor*.

Macrocentrus ancylicivora is the only larval parasite of importance in the Riverton district, as is clearly indicated in Table 2.

TABLE 2.—Relative abundance and importance of each species of larval parasite of the oriental peach moth present in peach orchards at Riverton, N. J., during the seasons of 1925 to 1928, inclusive, as determined by collections of twigs from orchards 2 to 6 years old ^a

Species	1925			1926			1927			1928			4 seasons (1925 to 1928)		
	Number of individuals emerged	Percentage of larvae parasitized	Percentage of all para- sites	Number of individuals emerged	Percentage of larvae parasitized	Percentage of all para- sites	Number of individuals emerged	Percentage of larvae parasitized	Percentage of all para- sites	Number of individuals emerged	Percentage of larvae parasitized	Percentage of all para- sites	Number of individuals emerged	Percentage of larvae parasitized	Percentage of all para- sites
<i>Macrocentrus ancylihora</i> Roh.	437	49.27	91.6	532	55.30	91.0	1,003	52.79	89.0	1,027	41.60	93.3	2,999	48.23	91.2
<i>Cremastus minor</i> Cush.	18	2.03	3.8				47	2.48	4.2	38	1.54	3.4	103	1.65	3.1
<i>Macrocentrus delicatus</i> Cress.				9	.94	1.5	51	2.69	4.5	25	1.01	2.3	85	1.36	2.6
<i>Cremastus forbesii</i> Weid.	17	1.92	3.6	25	2.60	4.3	1	.05	.1	2	.08	.2	45	.72	1.3
<i>Glypta rufiscutellaris</i> Cress.	3	.34	.6	17	1.77	2.9	5	.26	.4	4	.16	.4	29	.46	.9
<i>Ascopaster carpopapseae</i> (Vier.)	1	.11	.2				11	.58	.9	3	.12	.3	15	.24	.5
<i>Lixophaga variabilis</i> Coq.				2	.21	.3	1	.05	.1				3	.05	
<i>Pristomerus ocellatus</i> Cush.							3	.16	.3				3	.05	
<i>Apanteles harti</i> Vier.							2	.11	.2				2	.03	
<i>Bassus carpopapseae</i> Cush.										1	.04	.1	1	.02	
<i>Phanerotoma tibialis</i> Hald.	1	.11	.2										1	.02	.4
<i>Diocles obliteratus</i> (Cress.)							1	.05	.1				1	.02	
<i>Dibrachys</i> (<i>Tritoneptis</i>) <i>hemerocampae</i> Gir ^b							1	.05	.1				1	.02	
<i>Perilampus</i> sp. ^b							1	.05	.1				1	.02	
Parasitism of larvae by all species	477	53.78	100.0	585	60.81	100.0	1,127	59.32	100.0	1,100	44.55	100.0	3,289	52.89	100.0
Total oriental peach moths	410			377			773			1,369			2,929		

^a The collections in 1925 were from 2 orchards, in 1926 from 3 orchards, and in 1927 and 1928 from 4 orchards.

^b Secondary parasite.

SEASONAL PARASITISM

The total parasitism of larvae for each season by all species combined and the average parasitism for the four seasons are also given in Table 2. The seasonal parasitism was highest in 1926, when 60.81 per cent of the larvae collected in twigs were parasitized. The lowest parasitism occurred in 1928, when only 44.55 per cent were parasitized. An average of 52.89 per cent of all the larvae collected in peach twigs during the entire 4-year period was killed by various species of larval parasites.

In Table 3 the collections from the several orchards each week during a given season have been combined. This table gives the number of individuals which emerged, including both moths and parasites, the total percentage of parasitism by all species, the percentage of parasitism by *Macrocentrus ancylihora* alone, and the percentage by all other species combined, as they occurred in the weekly collections. In the collections made June 23 and 24, 1925, and July 15, 1926, as high as 95 per cent of the larvae were parasitized. The maximum parasitism in weekly collections in 1927 was considerably lower, 68 per cent, and occurred in the collections of

June 7 to 10 and of July 5 to 7. In the collection of July 3 to 5, 1928, there was a maximum parasitism of 72.1 per cent. In each instance indicated above, the percentage of parasitism by *M. ancylihora* alone also reached the maximum. The figures given in Table 3 further indicate the importance of *M. ancylihora* in the Riverton district.

TABLE 3.—Percentage of parasitism in individuals which emerged from weekly collections of peach twigs infested with larvae of the oriental peach moth at Riverton, N. J.

Collection dates	Number of individuals emerged	Percentage of larvae parasitized	Percentage of larvae parasitized by—		Collection dates	Number of individuals emerged	Percentage of larvae parasitized	Percentage of larvae parasitized by—	
			M. ancylihora	All other species				M. ancylihora	All other species
1925					1927				
May 18-21	58	67.2	67.2	---	May 11-13	7	57.1	42.8	14.3
May 26, 27	148	66.9	64.2	2.7	May 17-19	28	28.6	28.6	---
June 2, 3	130	36.9	36.9	---	May 24-26	153	59.5	59.5	---
June 9, 10	92	13.0	13.0	---	May 31-June 2	300	63.3	63.0	0.3
June 16, 17	98	24.5	23.5	1.0	June 7-10	399	68.2	64.4	3.8
June 23, 24	109	95.4	92.7	2.7	June 14-16	284	59.1	53.5	5.6
June 30, July 1	96	93.8	71.9	21.9	June 21-23	222	54.0	44.6	9.4
July 7, 8	41	73.2	58.6	14.6	June 28-30	218	52.3	28.0	24.3
July 14, 15	13	46.2	38.5	7.7	July 5-7	152	68.4	63.2	5.2
July 21, 22	6	33.3	33.3	---	July 12-14	89	51.7	44.9	6.8
July 27, 28	6	50.0	33.3	16.7	July 19-21	7	42.9	28.6	14.3
Aug. 2-6	8	50.0	37.5	12.5	July 26-28	10	10.0	10.0	---
Aug. 11, 12	14	21.4	14.3	7.1	Aug. 2-4	20	25.0	15.0	10.0
Aug. 18	10	20.0	20.0	---	Aug. 9-12	10	10.0	10.0	---
Aug. 25, 26	21	33.3	28.7	4.7	Aug. 17-19	1	---	---	---
Sept. 1	15	6.7	6.7	---	1928				
Sept. 8	17	5.9	5.9	---	May 16-19	32	6.3	6.3	---
Sept. 15	4	50.0	50.0	---	May 22-24	268	45.9	45.9	---
Sept. 22	1	---	---	---	May 29-June 1	453	50.1	49.9	2
1926					June 5-8	622	42.3	39.4	2.9
May 17-19	1	---	---	---	June 12-15	276	29.7	25.7	4.0
May 25, 26	34	47.1	47.1	---	June 19, 20	168	20.2	17.9	2.3
June 2, 3	87	40.2	40.2	---	June 26-28	173	40.5	29.5	11.0
June 8, 9	60	33.3	31.7	1.6	July 3-5	237	72.1	67.9	4.2
June 16, 17	60	11.7	11.7	---	July 10-13	149	69.1	64.4	4.7
June 23	64	9.4	7.8	1.6	July 17, 18	41	43.9	41.5	2.4
June 30	118	37.3	35.6	1.7	July 24, 25	36	11.1	5.6	5.5
July 6, 7	157	88.5	83.4	5.1	July 31-Aug. 2	2	50.0	50.0	---
July 15	107	95.3	84.1	11.2	Aug. 7-10	7	14.3	14.3	---
July 21	122	86.9	76.2	10.7	Aug. 14, 15	1	100.0	100.0	---
July 28	74	58.1	45.9	12.2	Aug. 21	4	---	---	---
Aug. 4	66	90.9	80.3	10.6					
Aug. 11	3	66.6	66.6	---					
Aug. 18	5	60.0	60.0	---					
Aug. 25	4	50.0	50.0	---					

With the exception of 1926 (when only one individual emerged from the first collection) parasitism varying from 6.3 to 67.2 per cent occurred in the first collection each season. It is thus apparent that the parasites emerge early enough in spring to attack the first larvae which enter the twigs. This was checked in the case of *Macrocentrus ancylihora* in the spring of 1928. It was found that these parasites began to emerge on May 11 after having hibernated in host larvae kept outdoors throughout the winter in a wire-screen cage hung on the south side of a pole, a method similar to that used by Peterson and Haeussler⁹ to determine the spring-brood emergence of oriental peach moths. This was five days earlier than the first oriental peach-moth larvae were found in twigs in the orchard (May 16).

⁹ PETERSON, A., and HAEUSSLER, G. J. DETERMINATION OF THE SPRING-BROOD EMERGENCE OF ORIENTAL PEACH MOTHS AND CODLING MOTHS BY VARIOUS METHODS. Jour. Agr. Research 37: 399-417, illus. 1928.

The most abundant parasitism occurred each season when larvae were most abundant in the twigs, as shown in Figure 3. The bars of this chart also indicate the number of moths, of *Macrocentrus ancylovora*, and of all other species of parasites combined which emerged from infested peach twigs collected weekly each season. The cross-hatched areas indicate the number of oriental peach moths,

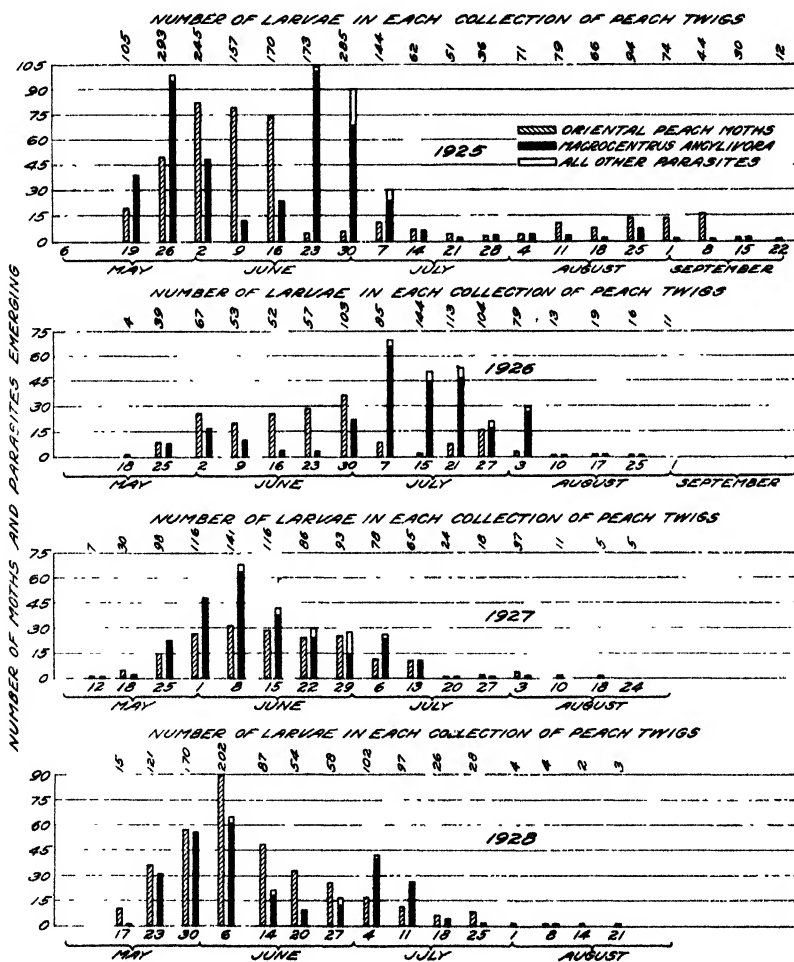


FIGURE 3.—Parasitism of oriental peach-moth larvae feeding in peach twigs at Riverton, N. J., 1925 to 1928, inclusive, as indicated by the number of individuals which emerged from infested twigs collected weekly in 15-minute periods

the solid black areas the number of *M. ancylovora*, and the white outlined areas the total number of parasites of all other species combined. The number of larvae taken in each collection is also given, and, as each collection was made during a 15-minute period, the figures indicate the relative intensity of infestation on each date of collection.

In 1925 a large population of first-brood host larvae was present in the twigs. These larvae were heavily parasitized in May, thus building up a strong population of parasites, chiefly *Macrocentrus ancylihora*, which in turn severely attacked the large second brood of host larvae. The heavy parasitism during the latter part of June and early July caused a decided decrease in twig infestation after the collection of July 7, but larvae were present in the twigs throughout August and until the collection of September 22. Parasitism after the second brood was comparatively low. There were five broods of host larvae at Riverton in 1925.

Host larvae of the first brood were not very abundant in the twigs in 1926, and parasitism of these larvae was low. Host larvae of the second brood were abundant in twigs throughout July and were heavily parasitized. Although twigs suitable for infestation were present in the orchards well into September, very few twigs were attacked after the collection of August 3. Only four broods of host larvae occurred at Riverton in 1926.

In 1927 the host larvae were abundant in the twigs from the collection of May 25 to the collection of July 13, and in every collection made during this period 50 per cent or more of the larvae were parasitized. This continuously heavy parasitism reduced the host population to such an extent that very few larvae were present in twigs after the middle of July. Four broods of host larvae occurred this season.

The situation in 1928 was, in some respects, similar to that in 1925. Larvae of the first brood were the most abundant and were heavily parasitized. Parasitism of larvae of the greatly reduced second brood was very severe early in July, and few host larvae were found in twigs after July 25, although a supply of twigs suitable for infestation was available until about August 10. There were five broods of host larvae in 1928.

PARASITISM OF LARVAE FEEDING IN FRUIT

Parasitism as high as 28 per cent by *Macrocentrus ancylihora* and 7 per cent by *M. delicatus* has been found in larvae feeding in peaches during the months of June, July, and August at Riverton. It is possible that many of these larvae were parasitized while feeding in twigs and that they abandoned the twigs later to complete their feeding in the fruit. From a large number of larvae cut from peaches from late August to early November in 1926, a total of 673 oriental peach moths and 10 *M. ancylihora* emerged the following spring. This was only 1.46 per cent parasitism, all by one species of parasite, in wintering larvae. These larvae probably fed almost entirely in the fruit.

From larvae collected in the fall of 1928 while feeding in quince fruit, five species of primary parasites and one secondary parasite emerged, as shown in Table 4. Of the 949 individuals which emerged, 40 were parasites, a parasitism of 4.21 per cent in larvae feeding in quince fruit at harvest time. *Phanerotoma tibialis*, which oviposits in the egg of the host and develops in the larva, was the most abundant species. The 24 individuals of this species and one of the two *Macrocentrus ancylihora* hibernated in the larvae of the host and did not emerge until the following spring. The other *M. ancylihora* and all the individuals of the remaining species emerged in the fall

of 1928. Of the 40 parasites which emerged from this material, 10 were *Glypta rufescutellaris*, whereas there were only 4 individuals of this species among 1,100 parasites obtained from larvae feeding in peach twigs collected earlier the same season. (Table 2.)

TABLE 4.—Parasitism of oriental peach moth larvae feeding in quince fruit at Riverton, N. J., 1928

Species	Number of individuals emerged		
	Trans-forming	Winter-ing	Total
<i>Phanerotoma tibialis</i> Hald.		24	24
<i>Glypta rufescutellaris</i> Cress	10		10
<i>Macrocentrus ancyliivora</i> Roh.	1	1	2
<i>Calliephialtes grapholithae</i> (Cress)	2		2
<i>Pristomerus ocellatus</i> Cushman	1		1
<i>Hemiteles tenellus</i> (Say)	1		1
Total parasites emerged	15	25	40
Oriental peach moths emerged	31	878	909
Total individuals emerged	46	903	949
Percentage of parasitism			4.21

* Secondary parasite.

PARASITISM AND MORTALITY OF ORIENTAL PEACH MOTHS IN COCOONS COLLECTED FROM UNDER QUINCE BARK

From a number of oriental peach moth cocoons collected under quince bark in the fall of 1928, only 74 individuals emerged, 15 of which, or 20.27 per cent, were parasites. (Table 5.) Eleven of the parasites were *Aenopler betulaecola*, which attacks the host in the cocoon, probably when it is a prepupa. One of these emerged in the fall of 1928, and 10 hibernated. All the other species represented attack the host while in the cocoon, with the exception of *Pristomerus ocellatus*, a parasite of the larva.

TABLE 5.—Parasitism and mortality of oriental peach moths in cocoons collected under quince bark at Riverton, N. J., 1928

Species	Number of individuals emerged		
	Trans-forming	Winter-ing	Total
<i>Aenopler betulaecola</i> Ashm.	1	10	11
<i>Centeterus ineptifrons</i> Gahan	1		1
<i>Ephialtes aequalis</i> (Prov.)		1	1
<i>Hoplocryptus incertulus</i> Cushman		1	1
<i>Pristomerus ocellatus</i> Cushman		1	1
Total parasites emerged	2	13	15
Oriental peach moths emerged	2	57	59
Total individuals emerged	4	70	74
Percentage of parasitism			20.27

Number of oriental peach-moth larvae dead 162

Number of oriental peach-moth pupae dead 15

Total individuals dead 177

Percentage of mortality 70.52

As shown in Table 5, 70.52 per cent mortality occurred in the larvae and pupae in the cocoons collected under quince bark. Most of these were apparently killed by a fungous disease which is common in oriental peach-moth larvae that hibernate in cocoons spun under bark.

SUMMARY

Fifty-seven species of primary parasites attacking the oriental peach moth in North America have been recorded thus far. Those of chief importance are the larval parasites *Macrocentrus ancyliivora* and *Glypta rufiscutellaris*, and the egg parasite *Trichogramma minutum*. Eight species of secondary parasites have also been found.

Records of the parasites of larvae occurring in the vicinity of Riverton, N. J., were obtained by making weekly collections throughout the season of peach twigs infested by the host larvae, and rearing these larvae to maturity. By trimming off the leaves and placing the portion of stems containing the larvae on apples in glass jars, 39.1 per cent more individuals were able to complete their development than when each larva was removed from the twig and placed on fruit.

Trichogramma minutum was the only parasite found at Riverton which develops entirely within the egg of the host. *Ascogaster carpocapsae* and *Phanerotoma tibialis* oviposit in the egg of the host but complete their development within the larva.

Fourteen species of parasites of larvae were found in larvae feeding in peach twigs at Riverton during the seasons of 1925 to 1928, inclusive. *Macrocentrus ancyliivora* is the only important parasite of larvae in the Riverton district. During the 4-year period, 91 per cent of all the parasites of larvae present were *M. ancyliivora*, and parasitism by this species alone averaged 48.23 per cent.

The seasonal parasitism by all species of parasites combined varied from 60.81 per cent in 1926 to 44.55 per cent in 1928, with an average of 52.89 per cent for the four seasons.

Parasitism as high as 95 per cent occurred in weekly collections of larvae in 1925 and 1926.

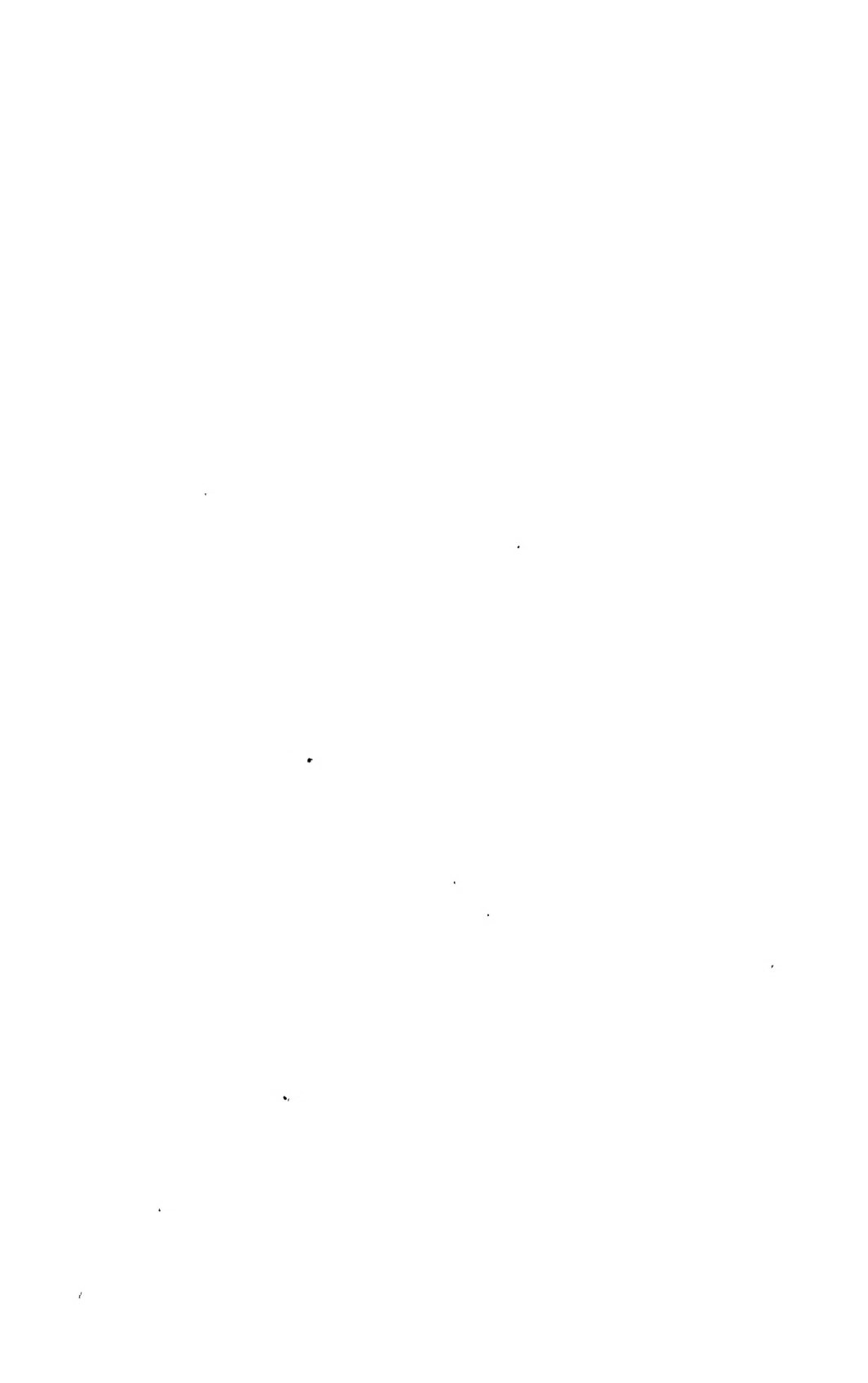
Macrocentrus ancyliivora emerges early enough in the spring to attack the first host larvae that feed in peach twigs.

The most abundant parasitism occurred each season when host larvae were most abundant in twigs. Parasitism of larvae of the first and second broods caused a decided decrease in the number of twigs infested each season.

Parasitism of 28 per cent by *Macrocentrus ancyliivora* and 7 per cent by *M. delicatus* occurred in larvae feeding in peach fruit in the early part of the season, but less than 2 per cent parasitism was found in wintering larvae collected while feeding in peaches in the fall.

About 4 per cent of the larvae collected while feeding in quince fruit at harvest time were parasitized, and *Phanerotoma tibialis* was the most abundant parasite. Parasitism by *Glypta rufiscutellaris* was much more abundant in these larvae than in larvae feeding in twigs.

Aenopler betulaecola was the most common parasite of the stages within the cocoon. High mortality, due to disease, occurred in oriental peach-moth larvae hibernating in cocoons spun under quince bark.



SHORT BRANCH, ANOTHER CHARACTER OF COTTON SHOWING MONOHYBRID INHERITANCE¹

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INTRODUCTION

Satisfactory evidence of monohybrid or simple Mendelian inheritance has been adduced for relatively few characters of the cotton plant. The following list of pairs of allelomorphs comprises the characters known to the writer for which there has been published fairly conclusive proof of monohybrid segregation in certain crosses.

Plant "crinkled dwarf"; plant normal (11).²

Leaf narrow-lobed ("okra"); leaf broad-lobed (4, 8, 20, 22, 23, 26, 27, 28, 32).

Leaf red; leaf green (4, 23, 26, 31, 32).

Corolla whitish; corolla yellow (1, 3, 7, 8, 12, 15, 17, 20, 21, 23, 24, 26).

Petal spot absent or weak; petal spot intense (1, 10, 13, 17, 18, 21, 24, 26).

Pollen cream-colored; pollen yellow (1, 2, 4, 14, 17, 26).

Seeds fuzzy; seeds naked or nearly so (1, 4, 7, 8, 17, 19, 21, 26, 29, 30).

Lint sparse; lint abundant³ (9, 20, 21, 29, 30).

A few other characters, in regard to which the evidence for the existence of a single pair of allelomorphs seems less conclusive or is conflicting, are: Color of the leaf pulvinus (1, 17, 21), color of the unripe boll (21), and color of the fuzz on the seed (4, 21).

Several of the characters in the first list have shown clear-cut monohybrid inheritance in crosses between nearly related forms but a more complicated type of inheritance in interspecific hybrids. A good example is spotless or weak-spotted petal as contrasted with full-spotted petal. When a full-spotted and a weak-spotted form of the same species were crossed, all plants of the F₂ population could readily be referred to one or the other group, and the departure of the respective numbers from a 3:1 ratio was insignificant (18). On the other hand, when the cross was between different species, e. g., full-spotted Egyptian or sea-island cotton (*Gossypium barbadense*) and spotless upland cotton (*G. hirsutum*), F₂ showed intergradation between the two classes, and separation of them was more or less arbitrary (13, 17).

An explanation of this difference in the behavior of crosses between related and unrelated forms has been offered by Harland (14, p. 399).

* * * species hybrids in cottons differ from varietal hybrids in the extent to which differences occur in modifying factors. In a species hybrid each main gene is apparently accompanied by a group of modifiers which have the effect of diluting the character in steps down to the recessive, and in certain cases may obscure entirely the distinction between dominant and recessive.

The argument is restated in another paper by the same author (16), as follows:

¹ Received for publication May 1, 1930; issued September, 1930.

² Reference is made by number (italic) to Literature Cited, p. 386.

³ The only well-supported instance of definite alternative inheritance of sparse lint as contrasted with abundant lint appears to be the one described by Thadani (29, 30). In this case there seemed to be close linkage with naked seed as contrasted with fuzzy seed, and it may be that only different expressions of a single gene are involved.

In varietal crosses there is segregation of the main gene alone, whereas in the interspecific cross there is segregation of modifiers which may partially, or in some cases entirely, obscure the distinction between dominant and recessive. Thus, there is no real difference in the mechanism of inheritance in interspecific from that which exists in varietal crosses.

The difference between crosses within a species and interspecific hybrids may extend to a reversal of dominance. Thus, in crosses between a naked-seeded and a fuzzy-seeded upland cotton or between a nearly naked seeded and a fuzzy-seeded Egyptian cotton the first-generation plants were all naked-seeded or nearly so, and the second generation segregated approximately in a ratio of 3 naked (or nearly so) to 1 fuzzy (19, 29, 30). But in crosses between upland cottons having the seeds completely fuzzy and Egyptian cottons having the seeds only partly covered with fuzz, the F_1 plants all had the seeds completely covered and F_2 showed an unbroken range between the two extremes (17, 29, 30).

DESCRIPTION OF THE CHARACTER "SHORT BRANCH"

A striking variant was discovered by Byron Reddin of Tempe, Ariz., in his field of Pima Egyptian cotton in 1924. This plant was characterized by having all its fruiting branches reduced to only one developed internode. Seeds produced by naturally pollinated flowers on this individual were planted at the United States field station at Sacaton, Ariz., in 1925, and approximately half of the nine plants that came up resembled the original variant. The others had fruiting branches with more than one internode, although shorter than in typical Pima plants. Evidently many of the ovules on the mother plant of 1924 had been cross-fertilized by pollen from the surrounding normal plants.

Flowers were bagged on a plant in this progeny which resembled the original variant, and from the resulting self-fertilized seeds a progeny of five individuals was grown in 1926. All of these plants bore only reduced (1 internode) fruiting branches; and the inbred progenies successively descended from one of them, comprising 8 individuals in 1927, 11 in 1928, and 4 in 1929, expressed the character uniformly. It may be concluded that the original variant of 1924 and the plant selected for selfing in 1926 were homozygous for fruiting branches reduced to one developed internode.

The fact that the cotton plant bears two kinds of branches, vegetative branches and fruiting branches, was pointed out by Cook (5, p. 13-31). Cook showed that the vegetative branches arise from either axillary or extra-axillary buds on the main stem, usually near its base. The fruiting branches arise only from extra-axillary buds, one of which is produced normally at each node of the main stem (primary fruiting branches) and one at each node of the vegetative branches (secondary fruiting branches). The flowers ordinarily develop from extra-axillary buds on the fruiting branches, one at each node. There are, however, many deviations from this general plan of development, as Cook has shown.

None of the fruiting branches of the plants grown at the Sacaton station had more than one developed internode, at the summit of which were borne from one to three flowers. Where only one flower was borne, the appearance was as if a greatly elongated pedicel or flower stalk had sprung directly from a node of the main stem. Where

one or two additional flowers appeared, they may have developed from accessory buds of the same internode, or possibly each represented an additional internode, so greatly shortened as not to be discernible.⁴ Occasionally the pedicels of two or three of these flowers were fused together (fasciated). Frequently two or three of these reduced fruiting branches arose from the same node of the main stem, the additional ones presumably by development from an axillary bud or from an axillary and an accessory bud, in addition to the single extra-axillary bud from which the fruiting branch at each node of the stalk normally develops.⁵ The shortened fruiting branches were more nearly erect than the branches of normal Pima plants. This difference was due perhaps to the absence of the mechanical stimulus exerted by the heavier load of bolls on normal branches.

The occasional occurrence of a small bractlike leaf on the reduced branch, such as may be seen near the top of the right-hand plant in Figure 1, proves that the structure can not be regarded as a simple pedicel. Similar occurrences in Egyptian cotton plants were noted by Cook (5, p. 24), who wrote:

Small bractlike leaves or stipules are occasionally present, even on straight stems, and sometimes the joint between the branch proper and the true stem or pedicel of the boll remains distinct, even when there are no leaves or stipules.

McLachlan (25, p. 11), referring to fruiting branches reduced to one or two internodes which sometimes appear to arise in an axillary position at the same node of the main stem with a normal, extra-axillary fruiting branch, states:

At the lowest nodes where such branches appear they normally bear a leaf opposite the flower bud, but at higher nodes the leaf may be abortive and the branch appear as a very long flower stem borne directly on the main stalk of the plant.

The young plants illustrated in Figure 1 have produced only single flower buds at the summits of the short branches, but otherwise they show very well the striking character which distinguishes the variant from the normal Pima type. The latter is illustrated in Figure 2, representing a plant of corresponding age with fruiting branches extending to five developed internodes. It will be noted that the normal plant bears a leaf as well as a flower at each node of the fruiting branch (fig. 2), while the shortened fruiting branches of the variant are leafless or practically so. (Fig. 1.)

INHERITANCE OF SHORT BRANCH IN A CROSS WITH NORMAL BRANCH

In 1926 emasculated flowers on a plant of a Pima family having normally developed fruiting branches were pollinated with pollen from a plant in the first inbred progeny of the short-branch family. The normal Pima parent was of a progeny representing the twelfth successive inbred generation. For 12 years this family (PH-8) and others descended from plant P-1, self-pollinated in 1914, had furnished the parents representing typical Pima in crosses made for studying

⁴ Referring to a condition sometimes found in the fruiting branches of the so-called "cluster cottons" (upland type), Cook states (5, p. 18): "The leaf buds that normally continue the growth of the branches are sometimes replaced by flower buds, or adjacent leaf buds may be aborted and fall off, so that the branch soon ends with a flower or a boll and no more joints can be added."

⁵ A similar situation is described by Cook (5, p. 16) as follows: "Sometimes the normal extra-axillary fruiting branch is also replaced by a single flower bud, so that three flower buds may appear to come from each of the nodes of the main stem instead of the more normal complement of a limb and a fertile branch."

the inheritance of various characters (17, 18). It had been under close observation during all this time and had shown no indication of the short-branch character. The same was true of the progenies



FIGURE 1 --Two individuals of the short-branch family of Pima cotton grown in 1928, representing the fourth generation of descent from the original selection. The fruiting branches are reduced to a single internode and are nearly erect. (Photographed July 31)

representing the thirteenth, fourteenth, and fifteenth inbred generations of family PH-8, grown in 1927, 1928, and 1929. In the 1928 progeny none of the 12 plants had fewer than five well-developed internodes on the longest fruiting branch. On the other hand,

the 11 individuals of the short-branch parental progeny grown in 1928 and the 4 individuals of the corresponding progeny grown in 1929 bore no fruiting branches having more than one internode.

An F_1 progeny of the cross was grown in 1927, but only two plants survived. Most of the fruiting branches on these plants were reduced



FIGURE 2.—A typical plant of Pima cotton (family PH-8) of the same age as the plants shown in Figure 1 grown in 1928. The longer fruiting branches have five well-developed internodes and are much less nearly erect than in the short-branch plants. (Photographed July 31)

to a single internode, but some of the branches on both plants had two well-developed internodes and on one of the plants a branch of three internodes was produced. The branches that had two or three internodes were more spreading, hence more nearly normal in direction, than the 1-internode branches.

An F_2 progeny of 48 individuals was grown in 1928 from seed produced by strictly self-pollinated (bagged) flowers on one of the F_1 plants. These plants were classified on the basis of the number of internodes of the fruiting branch which had the greatest number of internodes on the plant in question. The resulting frequency distribution is given in Table 1.

TABLE 1.—Classification of the F_2 progeny of the cross "normal branch \times short branch," 1928

Number of plants	Frequency distribution for number of internodes of the fruiting branch having the highest number on the plant in question						Classification		
	1	2	3	4	5	6	Short (1 internode)	Intermediate (2 or 3 internodes)	Normal (4 to 6 internodes)
48	14	20	8	1	2	3	14	28	6

The classification in the last three columns of Table 1 was made on the assumptions that all individuals having no fruiting branches with more than 1 internode were homozygous for short branch, that all individuals on which the longest fruiting branch comprised not fewer than 4 internodes were homozygous for normal branch, and that the remaining individuals, which resembled the F_1 plants in having not more than 2 or 3 internodes on the longest fruiting branch, were heterozygous. On the assumption of a 1:2:1 ratio, the numbers expected in a population of 48 are 12:24:12, while the numbers observed were 14:28:6. χ^2 for the departure from the assumed ratio is 4.000, and taking n ($n-1$) as 2, the value of P by Fisher's table of χ^2 (6, Table 3) is approximately 0.15. The indication, therefore, is that the short-branch character is conditioned by a single gene. The fact that the F_2 plants classed as intermediate resembled the two F_1 individuals grown in 1927 leads to the conclusion that there is no dominance in the heterozygous condition.

To test these assumptions, F_3 progenies were grown in 1929 from seed produced by flowers bagged on eight of the F_2 plants of 1928. Two of these represented the short-branch class, four the intermediate class, and two the normal-branch class. The classification of these F_3 progenies is given in Table 2.

With the exceptions noted in the footnote to Table 2, the diagnosis of the F_3 plants was easily made. It is believed that the classification given in the last three columns is accurate, since it was arrived at by considering the plant as a whole and laying not too much stress on the single criterion of number of internodes in the longest fruiting branch. In accepting this classification, it is seen that the two F_2 individuals selected as short branch and the two F_2 individuals selected as normal proved homozygous, there having been no segregation in their F_3 progenies. On the other hand, all four of the F_2 individuals which had been selected as intermediate were heterozygous, having produced F_3 progenies in which all three classes were represented.

The numbers of plants in the individual F_3 progenies are too small to give consequence to the ratios, taken singly. It is permissible, however, to combine them as one population, since they are all descended from a single cross and from a single self-fertilized F_1 plant.

On this basis, in a population of 56, there were 15 short-branch, 31 intermediate, and 10 normal-branch plants, whereas the numbers to have been expected with a 1:2:1 ratio are 14, 28, and 14. χ^2 of the departure from the expected ratio is only 1.537, and the indicated value of P is approximately 0.45. The chances therefore are about even that the departure is due to random sampling and would disappear if a larger population were available for classification.

TABLE 2.—*Classification of the eight F_3 progenies of the cross "normal branch \times short branch," 1929*

F ₃ progeny of F ₂ No	Number of plants	Classification of F ₃ parent	Frequency distribution of F ₃ for number of internodes of the fruiting branch having the highest number on the plant in question							Classification of F ₃ on the same basis as F ₂ ^a		
			1	2	3	4	5	6	7	Short	Intermediate	Normal
6	14	Short	14							14	0	0
14	15	do	14	1						15	0	0
5	14	Intermediate	7		3	2	1	1		7	5	2
8	14	do	4	1	1	4	3	1		4	6	4
11	15	do	3	4	4	2	1		1	3	11	1
15	13	do	1	1	6	2	2	1		1	9	3
21	14	Normal				4	6	4		0	0	14
35	14	do				1	8	5		0	0	14

^a With the following exceptions: All individuals in the segregating F₃ progenies 5, 8, 11, and 15 which had 4 internodes on the longest fruiting branch also bore typical short branches of 1 internode only. The same was true of the individual in progeny 15 which had 7 internodes on the longest fruiting branch, the branch in question having been abnormal and partly metamorphosed into a vegetative branch. These 11 plants were classed as intermediate and only those individuals in the 4 segregating progenies which had 5 or 6 internodes on the longest fruiting branch were classed as normal. None of the latter plants bore any short (1-internode) branches, and thus they resembled all of the plants in the normal nonsegregating progenies 21 and 35. In progeny No. 15 one plant had several branches of 2 internodes. Since the other branches had only 1 internode, and since the 2-internode branches were otherwise of short-branch character, it seems reasonable to classify this individual as short and to regard the progeny in which it was the only aberrant plant as homozygous for short branch, notwithstanding the fact that no fruiting branches of more than 1 internode have been observed on any of the inbred descendants of the original short-branch plant.

The evidence from the third-generation populations strengthens materially the conclusion that short branch originated by mutation of a single gene. It may be added that no indication of linkage of this gene with any other has yet been discovered. So far as has been observed, the inbred descendants of the original short-branch plant and the segregates of this class in F₂ and F₃ of the cross are quite fertile and do not differ from the normal Pima family in any characters not attributable to the gene for short branch.

SUMMARY

Few characters of the cotton plant are known to be inherited in a definitely alternative manner. An addition to the short list of such characters, short branch, is described in this paper.

A plant showing the character was discovered in 1924 in a field of Pima Egyptian cotton in Arizona. The fruiting branches of this plant were reduced to a single internode which was leafless or practically so. Frequently two or three of these branches appeared at the same node of the main stem. From one to three flower buds were borne at the summit of the single internode. The character has been expressed uniformly in the inbred descendants of the original short-branch plant during four generations.

A cross was made in 1926 between a plant of an inbred Pima family having normal fruiting branches comprising usually five or more internodes and one of the short-branch plants. Only two plants survived in the F_1 progeny of the cross, and these showed an intermediate condition, bearing fruiting branches of from one to three internodes.

In the second generation there was segregation into three classes—short branch, intermediate, and normal or long branch. The proportions of the several classes suggested a 1:2:1 ratio, indicating absence of dominance in the heterozygous condition.

Third generation progenies were grown in 1929, representing two individuals of each of the presumably homozygous classes in F_2 , and four individuals of the presumably heterozygous (intermediate) class. The short-branch and the normal F_2 individuals bred true, while all of the F_2 plants selected as being probably heterozygous produced segregating progenies. Taking the four segregating F_3 progenies as one population, the departure from a ratio of 1 short branch : 2 intermediate : 1 normal was insignificant.

The evidence seems complete that the expression of the character "short branch" depends upon a single gene and that there is no dominance in respect to this character.

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RELATION BETWEEN MOISTURE CONTENT OF THE WOOD AND BLUE STAIN IN LOBLOLLY PINE¹

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INTRODUCTION

It is common knowledge that green sap lumber will become badly discolored by staining fungi unless the moisture content of the wood is reduced by drying the lumber at a fairly rapid rate. At certain seasons of the year, varying in different regions of the country, the general weather conditions are distinctly unfavorable for drying lumber. During such seasons blue staining is inevitable unless the lumber is treated with chemicals that prevent the development of the staining organisms, or unless drying is speeded up artificially by the use of dry kilns. At all seasons of the year control of the moisture content of the lumber is the most important phase of blue-stain prevention.

Neither the upper limiting moisture content at which the staining fungi will grow nor the optimum moisture content for their growth is of immediate interest from a practical standpoint in handling lumber. The lower limiting moisture content is, however, of paramount importance. Münch³ determined that this lower limiting moisture content was approximately 28 per cent, based on the oven-dry weight of the wood, for *Pinus sylvestris* in Europe. The purpose of the present paper is to give the results of a series of accurately controlled tests to find the moisture content of the sapwood of one of the native American woods, loblolly pine (*P. taeda* L.), below which blue stain would not occur.

METHOD OF EXPERIMENTATION

The wood used for the tests came from a second-growth tree felled near Mize, Miss., in late November, 1925. A short log was cut from the bole at a point about 7 feet from the ground. The ends of the log were coated with heavy paint to retard moisture loss. The log was then shipped to Madison, Wis., where it was stored in the open for about four months until it was cut into test sticks. During this storage period little, if any, evaporation of moisture took place. In fact, the water in the wood froze and remained in a solid state until the log was cut in the mill. Except for a small central core of heartwood, the log was all sapwood. So far as could be determined, no infection with blue-stain organisms or wood-destroying fungi took place between the time of felling and the time of test.

The test sticks were prepared as follows: A bolt 2½ feet long was cut from the butt end of the log. The lower 6 inches of this bolt

¹ Received for publication May 2, 1930; issued September, 1930

² In cooperation with the Forest Products Laboratory, Forest Service.

³ MÜNCH, E. DIE BLAUPÄULE DES NADELHOLZES. *Naturw. Ztschr. Forst u. Landw.* 5: 531-73, illus., 1907; 6: 32-47, [297]-323, illus., 1908.

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were cut off and discarded. The freshly exposed lower end of the bolt was stained with a dye to facilitate the orientation of the individual boards as they came from the saw. The bolt was then quarter-sawed into nominal 1-inch boards. Every board was numbered as it came from the saw.

Each board was dressed in an accurate planer to a thickness of three-quarters of an inch. The sapwood of the boards was then laid off into test sticks, each of which was properly numbered. The test sticks were cut out carefully with a fine-tooth saw so that their width was as near three-fourths of an inch as possible. By this method a large number of sticks approximately 2 feet long and three-fourths by three-fourths inch in cross section were obtained.

These sticks were then cross piled openly in a conditioning room where the relative humidity was maintained at approximately 60 per cent. The relative humidity varied slightly, the lowest point reached being 58 and the highest 72 per cent. The temperature in the room remained close to 80° F., reaching a low point of 75° for only a short period. Preliminary tests showed that the wood dried so quickly under these conditions that after four days the moisture

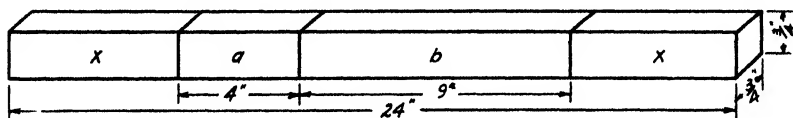


FIGURE 1 —Diagram showing the method used in cutting out the 4-inch moisture samples (a) and the 9-inch test sticks (b). The end pieces marked x were discarded

content had been reduced to approximately 14 per cent. This moisture content was known to be too low to support the growth of blue-stain fungi. Arrangements were therefore made to prepare some of the sticks for inoculation as soon as practicable after they were cut, and to remove others from the drying room for inoculation at short intervals after they had been placed therein. This procedure proved satisfactory. A series of test sticks with moisture content ranging from approximately 124 per cent to 14 per cent moisture content, oven-dry basis, was obtained.

The individual test sticks were cut from the 2-foot blanks, as shown in Figure 1. The middle part of each blank was laid off with the aid of a template into a 4-inch stick (fig. 1, a) to be used for determining moisture content and specific gravity, and a 9-inch test stick (fig. 1, b) to be used for inoculation. The 4-inch stick and the 9-inch stick were given the same identifying number and then cut. The pieces cut from the ends of the blank (fig. 1, x) were discarded.

The 4-inch and 9-inch sticks were placed in test tubes stoppered with cotton and then autoclaved 30 minutes at atmospheric pressure at a temperature of 100° C. After cooling, the 4-inch sticks were weighed. They were then dried to constant weight at 100° to 105° and weighed again. The percentage of moisture content, oven-dry basis, was later determined in the usual manner. Immediately after the dry weight was taken the dry samples were plunged into melted paraffin to prevent absorption of moisture. The volumes of the paraffin-coated sticks were determined by immersion in water in a finely graduated glass cylinder. The specific gravity of the sticks was

calculated on the basis of the oven-dry weight and the oven-dry volume.

It may be pointed out that a certain assumption is necessary in applying the results of the moisture content and specific gravity determinations made on the 4-inch sticks to the 9-inch test sticks. This assumption is that the moisture content of the smaller and larger pieces bearing like identifying numbers was the same after the samples had been autoclaved. The error in applying the specific gravity value of the smaller stick to the larger stick can be considered negligible. There may, however, have been a slightly larger loss in moisture content during autoclaving for the 4-inch stick than for the 9-inch test stick. The actual difference in the moisture content of any two like samples, however, is believed to have been slight, and it has been disregarded.

As soon as they had cooled, the 9-inch test sticks were inoculated with a strain of *Ceratostomella pilifera* which had been isolated in August, 1924, from the sapwood of loblolly pine. The infected wood came originally from North Carolina, but it is not known whether the log became infected there, during transit, or after it arrived at Madison.

The spores used for the inoculations were ascospores removed by a sterile needle from the tips of perithecia produced in a test tube on a malt-agar culture medium. The method of inoculation was as follows: A heavy suspension of spores was made in a small quantity of sterile water, and the spores were then transferred to the four sides of the test stick by means of a long platinum needle. An effort was made to distribute the spores from the top to the bottom of each side of each stick. No water other than the minute drops which clung to the platinum needle was added to any of the tubes either at the time of inoculation or later, so that it may be assumed that the inoculation method did not result in any significant increase in the moisture content of the inoculated sticks. The tubes containing the test sticks were capped with cotton pads held in place by pieces of cotton cloth tied down with string. They were placed in an upright position in a room with an average temperature of 78° F., and relative humidity of 82½ per cent.

Within 24 hours after inoculation the mycelium of the fungus could be observed along the track of the needle in the sticks having a moisture content high enough to support growth. Where the moisture content was distinctly favorable the development of the mycelium was rapid, and the sticks were completely enveloped in 21 days. The white mycelium soon became gray, and perithecia appeared in a few days. Where the moisture content was less favorable the mycelium was less luxuriant, the change to the typical gray color was retarded, and the formation of the perithecia was delayed or prevented. On sticks with relatively low moisture content the mycelium was concentrated toward the lower half of the stick, the upper part of the stick remaining clear.

EXPERIMENTAL DATA

Table 1 is made up of values for 48 apparently representative test sticks, calculated from the respective 4-inch moisture samples. Specific gravity, moisture content, and relative distribution of air,

wood substance, and water, expressed as percentage on a volumetric basis, are shown, together with the actual figures from which these values were determined. The values in columns 10 to 17 were obtained by the method described by Hartig⁴ and used by Münch⁵ in his description of the air, wood substance, and water relations in *Pinus sylvestris*. The figure 1.531 for the density of the actual wood substance in grams per cubic centimeter used in column 10, was specifically determined by Stamm⁶ from two samples of wood from the *P. taeda* under test, representing, respectively, material of low and high density. It differs but slightly from the value of 1.55 found by Hartig and used by Münch. Hartig's value 0.55, representing the percentage of water that would be absorbed by the wood substance, was accepted as approximately correct, and it was used to calculate the relative amounts of imbibition water and of free water shown in columns 16 and 17. These calculations give a somewhat higher value for the fiber saturation point than has been determined by experiment. By using the numbers under the column headings to represent the values in the columns themselves, excepting the values 1.531 and 0.55, the method of calculating the values may be explained as follows:

$$7 = 3 - 4; 8 = \frac{7}{4}; 10 = \frac{4}{1.531}; 11 = 10 + 7; 12 = 6 - (10 + 7); 13 = \frac{12}{6};$$

$$14 = \frac{10}{6}; 15 = \frac{7}{6}; 16 = 10 \times 0.55; 17 = 7 - 16.$$

The volume values in column 6 were calculated from data obtained from 52 additional 4-inch samples cut from blank sticks that were not used in any part of the preceding inoculation test. These sticks were prepared, however, at the same time and in the same machines as those used for the inoculation test. The 4-inch samples were laid out with the same template and cut with the same saw. Careful measurements and weighing showed them to be representative. Their volumes were determined by measuring the size of the samples with a steel scale graduated to 0.01 of an inch. The 4-inch samples had a range in moisture content from 102 per cent to 11.2 per cent. These values were plotted against the measured volumes and a curve drawn from which the average volume at any given moisture content could be read directly. The approximate average volume values were then inserted in column 6. It is obvious that this method is arbitrary. The volume in column 6 opposite laboratory number 35, for example, is not the actual volume of the 4-inch sample numbered 35, but the average volume of a 4-inch sample with an approximate moisture content of 25.4 per cent (column 8). The maximum error introduced by the use of an average instead of an actual volume value appears to be approximately 2 per cent at the wetter end of the series and 4 per cent at the drier end of the series. The difficulties in obtaining actual volume values for the sterilized test sticks at the beginning of the test are so great that the use of the arbitrary average value seems to be

⁴ HARTIG, R. ÜBER DIE VERTHEILUNG DER ORGANISCHEN SUBSTANZ, DES WASSERS UND LUFTRAUMES IN BAUMEN, UND ÜBER DIE URSACHE DER WASSERBEWEGUNG IN TRANSPIRIERENDEN PFLANZEN. 112 p. illus. Berlin. 1882. (Untersuchungen aus dem Forst-botanischen Institut zu München II.)

⁵ MÜNCH, E. Op. cit.

⁶ STAMM, A. J. DENSITY OF WOOD SUBSTANCE, ABSORPTION BY WOOD, AND PERMEABILITY OF WOOD. Jour. Phys. Chem. 33: [398]-414, illus. 1929.

justified. The error, whatever it may be, is carried over into columns 12, 13, 14, and 15 and into the graphic representation of the values in the last three of these columns. (Fig. 2.)

TABLE 1.—*Specific gravity, moisture content, and relative proportions of air, wood substance, and water in the test sticks at the beginning of the test*

Stick designation in Plates 1 and 2		Laboratory No.	Wet weight	Dry weight	Specific gravity, oven-dry basis	Volume at beginning of test	Water content ^a	Moisture content, oven-dry basis ^b	Moisture content, wet-wood basis ^b	Dry weight ^c 1.531	$\left(\frac{\text{Dry weight}}{1.531}\right) + (\text{water content})$ ^d	$\left[\frac{\text{Vol. c. c. at beginning of test}}{\left(\frac{\text{Dry weight}}{1.531} + (\text{water content})\right)}\right]$ ^e	Air, volume basis	Wood substance, volume basis	Water, volume basis	Imbibition water	Free water
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
		Gm.	Gm.		C. c.	C. c.	Per cent	Per cent		C. c.	C. c.	C. c.	Per cent	Per cent	Per cent	Gm	Gm
A.	1	39.14	17.50	0.522	37.0	21.64	123.7	55.3	11.43	33.07	3.93	10.6	30.9	58.5	6.25	15.35	
	2	40.00	18.47	.535	37.0	21.53	116.6	53.8	12.06	33.59	3.41	9.2	32.6	58.2	6.65	14.90	
B.	3	40.42	18.91	.556	37.0	21.51	113.8	53.2	12.35	33.85	3.14	8.7	33.4	58.1	6.80	14.71	
	4	38.11	17.85	.541	37.0	20.26	113.5	53.1	11.65	31.91	5.09	13.8	31.5	54.7	6.41	13.85	
C.	5	36.40	17.80	.524	37.0	18.60	104.5	51.2	11.62	30.22	6.78	18.3	31.4	50.3	6.39	12.21	
	6	38.00	18.75	.551	37.0	19.25	102.7	50.6	12.25	31.50	5.50	14.9	33.1	52.0	6.74	12.51	
D.	7	37.51	18.92	.573	37.0	18.59	98.3	49.6	12.35	30.94	6.06	16.4	33.4	50.2	6.80	11.79	
	8	38.40	19.60	.613	37.0	18.80	95.9	48.9	12.80	31.60	5.40	14.6	34.6	50.8	7.04	11.76	
E.	9	38.05	20.17	.630	37.0	18.48	91.6	47.8	13.16	31.64	5.36	14.5	35.6	49.9	7.24	11.24	
	10	36.91	19.56	.630	37.0	17.35	88.7	47.0	12.76	30.11	6.89	18.6	34.5	46.9	7.02	10.33	
F.	11	36.53	19.36	.569	37.0	17.17	88.7	47.0	12.65	29.82	7.18	19.4	34.2	46.4	6.96	10.21	
	12	37.10	19.81	.619	37.0	17.29	87.3	46.6	12.94	30.23	6.77	18.3	35.0	46.7	7.12	10.17	
G.	13	33.33	19.10	.538	37.0	14.38	74.5	42.7	12.47	26.70	10.30	27.8	33.7	38.5	6.80	7.37	
	14	33.70	19.50	.565	37.0	14.20	72.8	42.1	12.74	26.94	10.06	27.2	34.4	38.4	7.01	7.19	
	15	32.43	19.20	.565	37.0	13.23	68.9	40.8	12.54	25.77	11.23	30.3	33.9	35.8	6.90	6.33	
	16	30.02	18.04	.547	37.0	11.98	66.4	39.9	11.77	23.75	13.25	35.8	31.8	32.4	6.47	5.51	
H.	17	32.22	19.42	.598	37.0	12.80	65.9	39.7	12.69	25.49	11.51	31.1	34.3	34.6	6.98	5.82	
	18	31.38	19.90	.603	37.0	11.48	57.7	36.6	13.00	24.48	12.52	33.9	35.1	31.0	7.15	4.43	
	19	31.60	20.29	.588	37.0	11.32	55.8	35.8	13.24	24.55	12.44	33.6	35.8	30.6	7.28	4.06	
	20	27.63	18.41	.558	37.0	9.22	50.1	33.4	12.03	21.25	15.75	42.6	32.5	24.9	6.62	2.60	
I.	21	29.53	19.68	.596	37.0	9.85	50.1	33.3	12.85	22.70	14.30	38.7	34.7	26.6	7.06	2.79	
	22	22.80	15.62	.504	37.0	7.18	46.0	31.5	10.20	17.38	19.62	53.0	27.6	19.4	5.61	1.57	
	23	24.21	16.59	.510	37.0	7.62	45.9	31.5	10.83	18.45	18.55	50.1	29.3	29.6	5.96	1.66	
J.	24	27.00	18.57	.563	37.0	8.43	45.4	31.2	12.14	20.57	16.43	44.4	32.8	22.8	6.68	1.75	
	25	27.80	19.37	.587	37.0	8.43	43.5	30.3	12.65	21.08	15.92	43.0	34.2	22.8	6.96	1.47	
	26	26.92	19.28	.567	37.0	7.64	39.6	28.4	12.63	20.24	16.76	45.3	34.0	20.7	6.93	.71	
K.	27	24.72	18.24	.570	37.0	6.48	35.5	26.2	11.90	18.38	18.62	50.3	32.2	17.5	6.54	-.06	
	28	22.10	16.90	.504	36.9	5.20	30.8	23.5	11.04	16.24	20.66	56.0	29.9	14.1	6.01	-.87	
L.	29	23.03	17.93	.532	36.9	5.10	28.4	22.1	11.70	16.80	20.10	54.6	31.7	13.8	6.44	-1.34	
	30	22.00	17.17	.536	36.8	4.83	28.1	22.0	11.21	16.04	20.76	56.4	30.5	13.1	6.16	-1.33	
	31	23.35	18.36	.574	36.8	4.99	27.2	21.4	12.00	16.99	19.81	53.8	32.6	13.6	6.60	-1.61	
M.	32	23.91	18.95	.557	36.8	4.90	26.2	20.7	12.38	17.34	19.46	52.9	33.6	13.5	6.61	-1.85	
	33	22.92	18.21	.560	36.7	4.71	25.9	20.5	11.90	16.61	20.09	54.8	32.4	12.8	6.54	-1.83	
	34	21.67	17.24	.530	36.7	4.43	25.7	20.4	11.25	15.68	21.02	57.2	30.7	12.1	6.19	-1.76	
N.	35	22.65	18.07	.548	36.7	4.58	25.4	20.2	11.80	16.38	20.32	55.4	32.1	12.5	6.49	-1.91	
	36	23.67	19.90	.591	36.7	4.77	25.2	20.2	12.35	17.12	19.58	53.4	33.6	13.0	6.80	-2.03	
O.	37	23.00	18.44	.578	36.6	4.56	24.7	19.8	12.05	16.61	19.69	54.6	32.9	12.5	6.82	-2.06	
	38	24.23	19.52	.622	36.6	4.71	24.1	19.4	12.75	17.46	19.14	52.3	34.8	12.9	7.01	-2.30	
	39	24.04	19.39	.570	36.5	4.65	24.0	19.3	12.65	17.30	19.2	52.6	34.7	12.7	6.96	-2.31	
P.	40	23.17	18.69	.623	36.5	4.48	24.0	19.3	12.20	16.58	19.82	54.3	33.4	12.3	6.71	-2.23	
	41	24.28	19.75	.598	36.3	4.53	22.9	18.7	12.90	17.43	18.67	52.0	35.5	12.5	7.10	-2.57	
Q.	42	19.88	16.21	.514	36.3	3.67	22.6	18.5	10.59	14.26	22.04	60.7	29.2	10.1	5.82	-2.15	
	43	23.06	18.91	.610	36.3	4.15	22.0	18.0	12.35	16.50	19.80	54.6	34.0	11.4	6.80	-2.65	
R.	44	19.95	16.49	.492	36.1	3.45	21.0	17.3	10.76	14.22	21.58	60.6	29.8	9.6	5.82	-2.46	
	45	20.90	17.31	.525	36.0	3.59	20.7	17.2	11.30	14.89	21.11	58.6	31.4	10.0	6.22	-2.63	
S.	46	21.81	18.58	.562	35.4	3.23	17.4	14.8	12.14	15.37	20.03	56.6	34.3	9.1	6.68	-3.45	
	47	22.46	19.55	.611	34.9	2.91	14.9	12.97	12.77	15.68	19.22	55.1	36.6	8.3	7.02	-4.11	
T.	48	22.30	19.56	.631	34.8	2.74	14.0	12.3	12.78	15.52	19.28	55.4	36.7	7.9	7.03	-4.29	

^a Equivalent to volume of water in cubic centimeters (temperature change disregarded).

^b Computed from adjoining 4-inch segments.

^c Equivalent to volume of wood substance in cubic centimeters.

^d Equivalent to volume of wood substance plus volume of water in cubic centimeters.

^e Equivalent to volume of air in cubic centimeters.

The points plotted on Figure 2 represent the values on a volumetric basis for the percentages of air, wood substance, and water that are given in Table 1. The curves were drawn by inspection. Two sets of moisture-content percentage values based on weight are listed on the x axis, and the percentage of moisture content based on volume is indicated on the y axis. The method of plotting used makes the relation among the three ways of stating moisture content fairly clear. However, since there may be many readers who are more or less

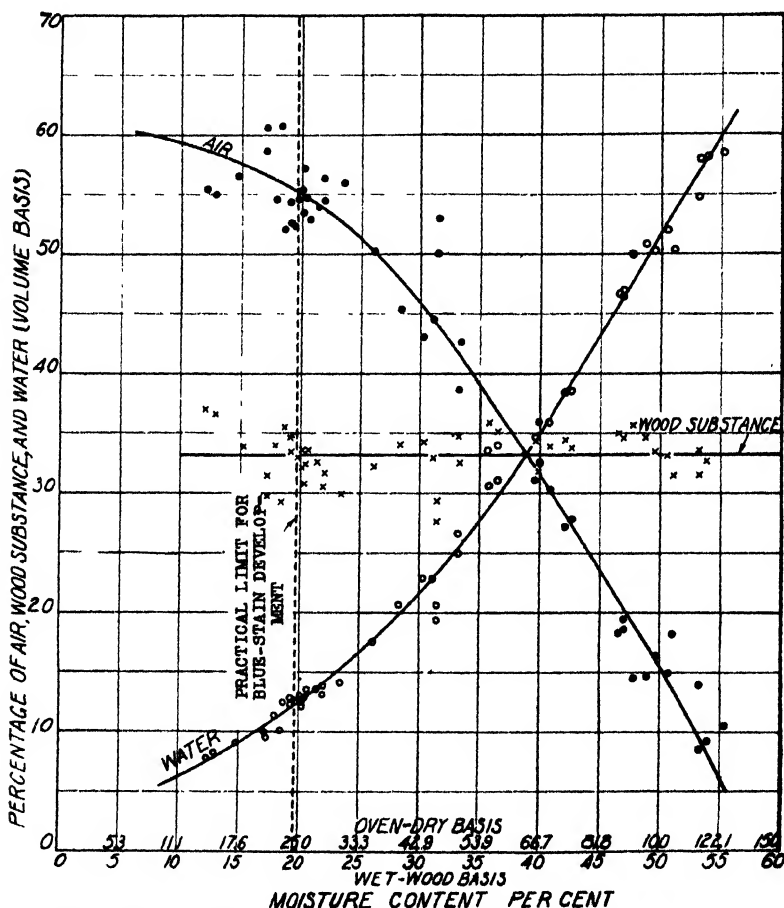


FIGURE 2.—Relative volumetric distribution of air, wood substance, and water in the test sticks at the beginning of the test

unfamiliar with the two more common methods of expressing moisture content on a weight basis, it has been thought best to include Figure 3. The curves in this figure show at a glance the relation between moisture-content percentages based on the weight of the oven-dry wood and moisture-content percentages based on the weight of the wet wood.

Table 2 gives a general description of the test sticks at the end of the test. The laboratory number and moisture content data are the

same as in Table 1. Tables 1 and 2, Figure 2, and Plates 1 and 2 should be studied together. Moisture content interpolations can be made with the help of Figure 3.

TABLE 2.—Results of the experiments on the inoculation of loblolly pine of different percentages of moisture content with a strain of *Ceratostomella pilifera*

Stick designation in Plates 1 and 2	Laboratory No	Moisture content, oven-dry basis, at beginning of test ^a	Duration of test ^b	Appearance at end of test	
				Surface	Interior
		Per cent	Days		
A	1	123.7	36	Covered with mycelium, abundant perithecia	Completely stained, dark.
	2	116.6	36	do	Do.
B	3	113.8	36	do	Do
	4	113.5	21	do	Do
C	5	104.5	35	do	Do
	6	102.7	36	do	Do.
D	7	98.3	35	do	Do
	8	95.9	35	do	Do.
E	9	91.6	36	do	Do
	10	88.7	35	do	Do.
F	11	88.7	35	do	Do
	12	87.3	35	do	Do
	13	74.5	34	do	Do.
G	14	72.8	34	do	Do
	15	68.9	34	do	Do.
	16	66.4	33	do	Do
H	17	65.9	34	do	Do
	18	57.7	34	do	Do.
	19	55.8	30	do	Do.
I	20	50.1	33	do	Do.
	21	50.1	33	do	Do
	22	46.0	22	do	Do
J	23	45.9	22	do	Do
	24	15.4	33	Covered with white mycelium	Do.
	25	43.5	31	do	Do.
K	26	39.6	33	Covered with light-gray mycelium	Do
	27	35.5	22	do	Do
	28	30.8	22	Lower 8 1/4 inches covered with mycelium	Bottom 3 inches completely stained, medium
L	29	28.4	22	Lower 8 inches covered with mycelium	Bottom 6 1/2 inches completely stained, medium.
	30	28.1	22	Lower 7 inches covered with mycelium	Bottom 7 inches completely stained, medium.
	31	27.2	22	Lower 7 1/2 inches covered with mycelium.	Bottom 2 1/2 inches completely stained, medium.
M	32	26.2	22	Lower 6 1/2 inches covered with mycelium	Bottom 1 1/2 inches completely stained, medium.
	33	25.9	23	Lower 2 inches covered with mycelium	Bottom 2 inches completely stained, medium.
	34	25.7	22	Lower 2 1/2 inches covered with mycelium.	Bottom 2 inches completely stained, light.
N	35	25.4	22	Lower 4 inches covered with mycelium.	Bottom 1/2 inch completely stained, light.
	36	25.2	22	Lower 3 inches covered with mycelium	Bottom 3/4 inch completely stained, light.
O	37	24.7	22	Lower 1 1/4 inches covered with mycelium	Bottom 2 inches completely stained, light.
	38	24.1	22	Lower 1 inch covered with mycelium.	Bottom 1 inch completely stained, light.
P	39	24.0	23	No mycelium evident.	Unstained.
	40	24.0	22	White and gray mycelium on bottom 1/2 inch	Do.
	41	22.9	22	White and gray mycelium on bottom 1 inch.	Do.
Q	42	22.6	21	do	Do.
	43	22.0	22	do	Bottom 1/2 inch completely stained, light.
R	44	21.0	21	White mycelium on bottom 1 1/2 inch.	Unstained.
	45	20.7	21	do	Do.
S	46	17.4	32	Scant mycelium on bottom 2 1/2 inches.	Do.
	47	14.9	32	Scant mycelium on bottom 1/2 inch	Do.
T	48	14.0	32	do	Do.

^a Computed from adjoining 4-inch segments.

^b Two experiments were run; the first closed after 30 to 36 days and the second after 21 to 22 days, a period found sufficient for complete interior staining.

The distribution of moisture in nine representative test sticks at the end of the test is shown in Table 3.

TABLE 3.—*Distribution of moisture (per cent) in some of the test sticks at the end of the tests*

Laboratory No.	Moisture content of 1-inch segments of test sticks, oven- dry basis									Moisture content of whole stick, oven- dry basis.			Duration of test (days)
	Bottom segment 1	Seg- ment 2	Seg- ment 3	Seg- ment 4	Seg- ment 5	Seg- ment 6	Seg- ment 7	Seg- ment 8	Top segment 9	At be- ginning of test ^a	At end of test ^b	Reduction during test ^c	
9453.	23.9	23.1	22.4	20.0	20.8	19.7	18.4	15.0	14.8	22.6	19.8	2.8	21
9416	37.6	41.1	39.3	39.0	38.6	39.2	40.0	38.5	40.1	45.9	39.3	6.6	22
9415	32.3	35.4	35.5	33.5	34.5	34.0	32.6	30.6	27.4	35.5	32.8	2.7	22
8437.	40.6	42.8	44.5	42.4	39.7	42.6	40.2	42.0	39.6	55.8	41.6	14.2	30
8433	39.0	40.0	37.8	36.4	37.5	36.2	34.5	35.0	30.6	50.1	36.3	13.8	39
9475	29.0	28.2	26.6	25.4	22.9	22.3	20.3	17.6	16.0	25.7	23.1	2.6	22
9414	34.4	32.1	31.1	27.5	27.8	26.8	23.7	20.6	17.4	27.2	26.7	.5	22
9423	32.7	33.5	31.6	30.6	29.1	26.8	24.5	21.2	17.1	30.8	27.5	3.3	22
9413	45.8	43.9	42.8	41.0	43.0	42.7	41.6	42.3	39.1	46.0	42.5	3.5	22

^a Assumed to be same as that found in an adjacent 4-inch segment from the same stick.

^b Determined from total dry weight and total water from the 9 segments

^c Difference between the two preceding figures.

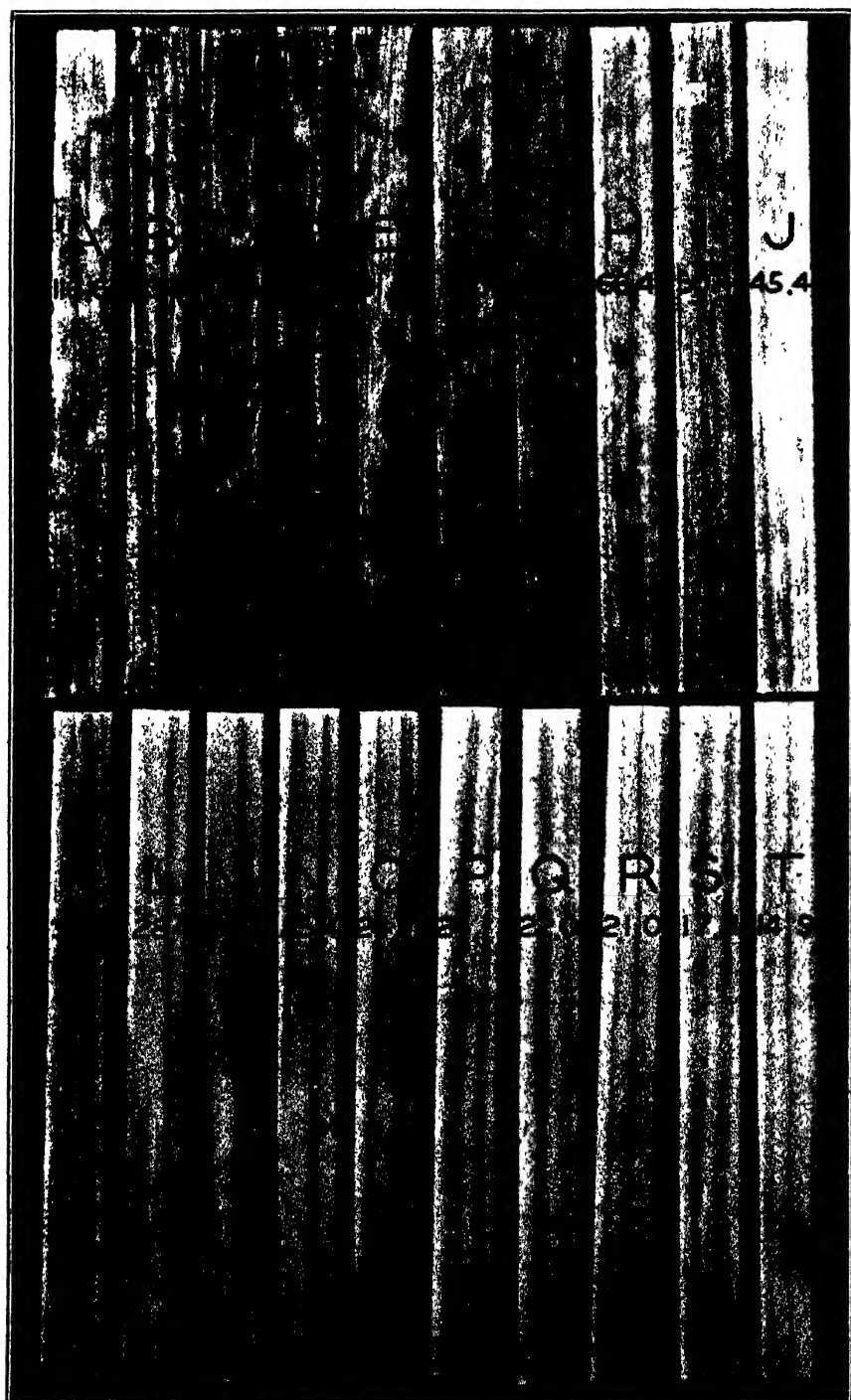
DISCUSSION OF RESULTS

Sticks A to G in Plate 1 are covered with gray mycelium. Sticks H to K are covered with mycelium that is not so dark. Stick L shows approximately 1 inch of clear wood at the top, and from stick M to stick R there is a rapid falling off in the height reached by the mycelium. On stick R the mycelium is reduced to little more than a trace at the very bottom. The moisture content values of sticks O to R at the beginning of the test ranged from 24.7 per cent down to 21.0 per cent, oven-dry basis. Stick S showed a scant white mycelium at its base which disappeared when the stick was handled. Stick T showed a white mycelium at its base, so tenuous and sparse that it had disappeared before the end of the experiment. (Pl. 1.) Data on the distribution of air, water, and wood substance in these sticks are given in Table 1.

After the negative for Plate 1 was taken the sticks were sawed lengthwise. The freshly exposed surface of one-half of each stick was then planed, and the planed sticks were arranged (pl. 2) in the same order as they appear in Plate 1. This method allows easy comparison between the exterior and the interior appearance of the sticks. It is evident from Plate 2 that sticks A to G were heavily stained throughout. Beginning with stick H there is a steady diminution in the amount of stain, with the last visible trace of discoloration showing at the bottom of stick Q. On the evidence presented in these two plates it might be concluded that the lower limiting moisture content, below which the staining organism would not develop, was approximately 21 to 22 per cent, oven-dry basis. Table 2 shows that development of mycelium was very evidently retarded on sticks which had a moisture content at the beginning of the test of 24 per cent or less. The data in Table 3 help to explain these results.



Twenty representative test sticks at the end of the test, arranged in series to show the relation between moisture content and the development of mycelium on the surfaces of the sticks. The moisture-content values of the sticks at the beginning of the test are marked on them. Complete data are shown in Tables 1 and 2



The same 20 sticks that appear in Plate 1, split to show the stain inside of the test sticks

Stick 42, for example, had a moisture content of 22.6 per cent at the beginning of the test. At the end of the test the moisture content was found to be 19.5 per cent; that is, there was a loss of 3.1 per cent during the 21 days the test was run. Examination of adjoining 1-inch segments of the stick showed that there was a definite moisture gradient in the stick with a high moisture content of 23.9 per cent in the bottom inch and a low moisture content of 14.8 per cent in the top inch. At just what point in the course of the test this gradient was established is not known. It can be safely assumed that the gradient was not constant. How the gradient was established seems reasonably clear. The loss in moisture content shown in Table 3 must have been

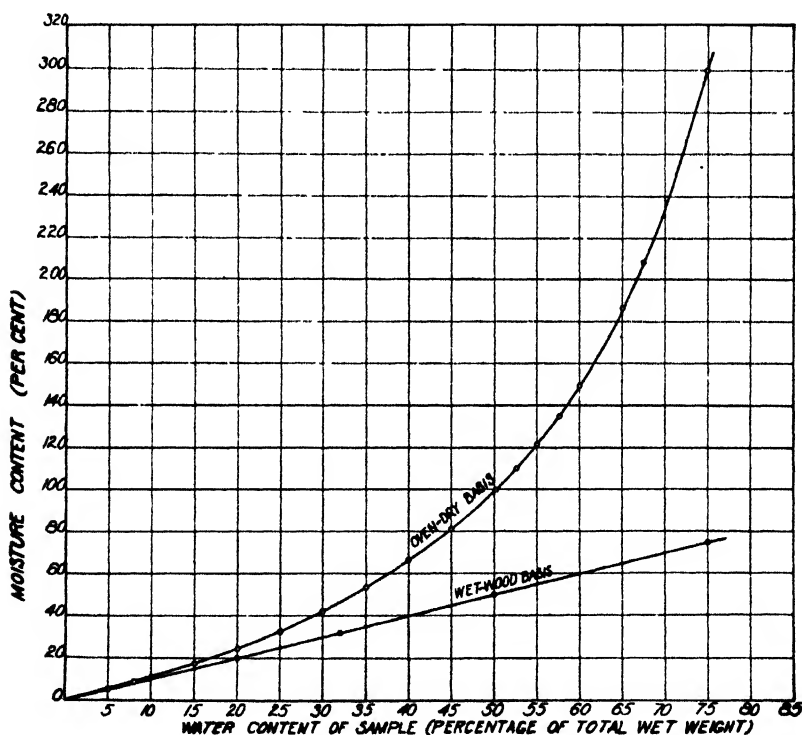


FIGURE 3 —Relation between moisture content percentages based on the weight of the oven-dry wood, and moisture-content percentages based on the weight of the wet wood

caused by evaporation through the cotton pad and cloth. One would therefore expect the upper end of the stick to be somewhat drier than the lower end. There is another reason, however, for the higher moisture content of the lower end. Very slight changes in temperature caused a condensation of moisture on the inside of the tubes. The moisture collected in drops and ran to the bottom of the tube. Daily observation showed that this moisture was absorbed by the wood at the lower end of the stick. The two processes, evaporation from the top of the stick and absorption at the bottom of the stick, resulted in the establishment of the moisture gradient. In stick 42 the moisture content near the bottom at the beginning of the test or soon there-

after may have been several points in excess of the 23.9 per cent found at the end of the test. The moisture content was evidently high enough to support the growth of the mycelium for a time, but not high enough to result in staining the interior of the stick. (Table 2.)

Table 3 shows that the gradient was sometimes steeper than in stick 42. In stick 34, for example, each of the lower 4 inches of the stick had a moisture content in excess of 25 per cent at the end of the test, although the moisture content for the whole stick was only 22.7 per cent. Table 2 shows that the lower 2½ inches of this stick were covered with mycelium and that the lower 2 inches of the stick were stained inside. At the beginning of the test this stick had a moisture content of 25.7 per cent. It may be compared with stick N in Plates 1 and 2.

In sticks similar to stick 20 or 23 (Table 3), which are comparable to sticks I and J in Plates 1 and 2, and in sticks with a still higher moisture content the mycelium acts as a sort of wet blanket covering the surface, and in such cases moisture gradients are not likely to be established in tests running for so short a period as the duration of these tests.

CONCLUSIONS

From the tests it appears that the sapwood of *Pinus taeda* at a moisture content of 24 per cent or less, oven-dry basis, will not support sufficient growth of this strain of *Ceratostomella pilifera* to produce staining of the wood. At a moisture content of 24 per cent, oven-dry basis, which is equivalent to 19.3 per cent on the wet-wood basis, approximately 53.5 per cent of the volume of the wood is air, 35 per cent is wood substance, and 12.5 per cent is water, assuming reasonable accuracy in the methods used for determining the values shown in Table 1. The dotted vertical line at 24 (oven-dry scale) in Figure 2 marks the limiting conditions described above. A moisture content of 24 per cent should not be considered as the absolutely fixed limit below which blue stain will not occur. It may be assumed, however, that the lower limiting value is somewhere near 24 per cent.

Münch⁷ concluded that when air made up 61 per cent of the volume and when the wood substance lacked 7 per cent (oven-dry weight basis) of being saturated the development of *Ceratostomella coerulescens* was practically inhibited in *Pinus sylvestris*. The moisture content, oven-dry basis, calculated for these conditions from the values given in his Table 1 was 28. He says, however, that the fungus developed in the lower third of his test block and that the upper part of the block remained unstained. There is at least a possibility, judging from the results of the tests on *P. taeda*, that the lower part of the block used by Münch also had a moisture content in excess of 28 per cent. At any rate he mentions the fact that evaporation from the lower part of the block was somewhat retarded. Considering the possible effect of the difference in species of wood and the strain of fungus used, the results obtained on *P. taeda* check very well with the results on *P. sylvestris*.

From the growth reactions of *Ceratostomella pilifera* in culture on *Pinus taeda* in the laboratory, and from field observations in the southern pine area, it appears very probable that blue-stain fungi are

⁷ MÜNCH, E. Op. cit.

not likely to grow if the moisture content of the outside layers of cells is 24 per cent or below, provided the relative humidity is low. The moisture content of the interior wood might conceivably be higher than 24 per cent for a while at least; but if the moisture content of the outside of the wood is too low to support growth, a reduction in the amount of blue-stain infection is certainly possible. In excessively damp or rainy weather the outside layers of cells might have a moisture content of 25 per cent or more although the interior of the wood remained for a time somewhat drier. Under such conditions it is conceivable that blue-stain fungi might start growing. Unless the rainy weather were prolonged, as it is, of course, during the regular rainy season, the outside layers of cells would soon dry to below 24 per cent and the growth of the organisms would be stopped.

Attention is particularly drawn to the fact that the results presented in this paper were obtained on sapwood that had been submitted to a temperature of 100° C. for 30 minutes. Such steamed wood may be slightly different from sapwood that has not been so treated, due to the volatilization of some fractions of the resinous materials in the wood. No tests were made to determine this point. To avoid serious mold contaminations one is practically compelled to use wood that is disinfected at least superficially. The attempt was made in these tests to reduce the heat treatment to the shortest practicable period. The inoculation results are believed to approach those that would have been obtained on unsterilized *Pinus taeda* wood with an approximately equivalent moisture content, but no supporting data can be presented.

Field observations indicate some difference in the staining reactions of the different southern pines. For example, in certain sections it is commonly held that shortleaf pine stains more quickly and more severely than longleaf pine. If this be generally true, there appears to be considerable justification in applying the results of the tests on *Pinus taeda* in control experiments on the southern pines. Additional laboratory tests on these other pines with wood cut at various seasons of the year are necessary before it can be said more definitely what is the low moisture content that will inhibit the growth of blue-stain fungi in each species of wood. It seems wisest, therefore, to set a figure of 20 per cent moisture content as the practicable working limit and to consider the difference between this 20 per cent and the 24 per cent deduced from the tests as a reasonable margin or factor of safety.

THE ADSORPTION OF THE ANIONS OF ACID DYES BY SOIL COLLOIDS¹

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INTRODUCTION

Previous studies (2)² have shown that the capacity of soil colloids for adsorbing or exchanging inorganic cations varies roughly with the molecular silica-sesquioxide ratio of the colloid. This parallelism has been explained on the ground that the exchangeable bases are held in some sort of combination with acidic, silica, or aluminosilicate radicals and that the reactive surface of the colloid occupied by these radicals varies roughly with the gross content of SiO₂ (1, p. 579). It would seem to follow that those colloids having low silica-sesquioxide ratios and low exchangeable base capacities should have more or less aluminum or iron in the surface and that these colloids should therefore have appreciable adsorptive capacities for anions.

This idea that part of the surface of a soil colloid should be capable of combining with cations and part capable of combining with anions is not wholly supported by the available data. Although different soil colloids have appreciable and varying capacities for adsorbing cations from both neutral salt and basic dye solutions, they seem to have little if any capacity for adsorbing simple anions, such as Cl, NO₃, and SO₄, at least from neutral salt solutions. On the other hand, adsorptions of the complex anions of acid-dye solutions have been frequently reported.

In case anions of acid dyes are adsorbed, it would seem that the comparative capacities of a soil colloid for adsorbing acid and basic dyes should furnish a better characterization of the colloid than the single determination of the adsorption of a basic dye. It is therefore important to determine whether there is an acid dye that is appreciably adsorbed by soil colloids, quite aside from any evidence that may be afforded regarding the nature of the colloid surface. An investigation of the adsorptions of several acid dyes is described in this paper.

REVIEW OF LITERATURE

The idea that the adsorptive capacities of soil and other inorganic colloids for different dyes would throw light on the constitution of the materials is far from new. Sjollesma (15) suggested in 1905 that the extent to which soil colloids adsorb methyl violet, naphthol yellow, Congo red, and alizarin would indicate the presence of silicic acid, hydrous aluminum oxide, and aluminum silicate, since artificial gels of these compounds adsorb these dyes in different degrees. However, he gave no data on the adsorption of soils. Later Hundeshagen (11) showed that the adsorption of different dyes helped to identify

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² Reference is made by number (*italic*) to Literature Cited, p. 413.

certain minerals. The use of dyes for indicating the nature of the adsorbent is also suggested by the work of Michaelis and Rona (13), Bechhold (6), Testoni (16), and Birutowitsch (7) with artificial gels. In these investigations it was found that silica gels adsorbed only basic dyes, whereas alumina and iron gels adsorbed only acid dyes. Recently, Croucher (8) showed that adsorption of the acid dye, Biebrich scarlet, by an artificial hydrous alumina gel increased with increasing acidity, while adsorption of the basic dye, iodine green, decreased with increasing hydrogen-ion concentration, the minimum adsorption of the combined dyes occurring at a pH of 6.5, the isoelectric point of the gel. Since soils high in alumina and iron showed a similar behavior, he suggested that a marked change in the proportions in which these dyes are adsorbed above and below pH 6.5 constitutes a test for the presence of hydrous alumina in soils.

Observations on the adsorption of acid dyes by soil materials have been made by several investigators, although most quantitative studies of dye adsorption by soils (10) and clays (3, 4) have been conducted with the two basic dyes methylene blue and malachite green, which are highly adsorbed. Way (17) states that clays remove the coloring matter from cochineal solutions. According to Rohland (14), adsorption of the acid dye aniline blue by soils is comparable to that of the basic dyes crystal violet and Victoria blue; adsorption of the acid dyes orange and metanil yellow is also appreciable but much less than that of aniline blue. Bechhold (6) found that a clay and a permutite both adsorbed methylene blue strongly but took up none of the acid dye trypan blue and very little of the acid dye toluene red. Data on the adsorption of diamine sky-blue are given by Beaumont (5) for four soils. The quantities of this acid dye adsorbed were appreciable, but much less than the quantities of the basic dye methylene blue. Michaelis and Rona (13) report that a kaolin which adsorbed several basic dyes strongly did not adsorb any of the acid dyes potassium eosinate and ammonium picrate, but did adsorb some of the more colloidal dyes, Congo red and diamine fast red. Wilkinson and Hoff (20) give the adsorptions of six soils for two basic dyes and one acid dye, diamine blue 3 B, with and without the addition of acid. All dyes were appreciably adsorbed in the absence of acid, but the adsorption of diamine blue 3 B was especially increased by acid. Birutowitsch's data show that a "Floridin" soil adsorbed the acid dyes night blue, eosin, and Congo red, but not azo blue. Croucher (8) reports a marked adsorption of Biebrich scarlet (an acid dye) by soils high in iron and aluminum from solutions below pH 6.5. These results on the adsorption of acid dyes may be summarized as follows: Certain investigators found trypan blue, toluene red, potassium eosinate, and ammonium picrate not adsorbed by soils or similar materials; other investigators report adsorptions of cochineal, aniline blue, orange, metanil yellow, diamine sky blue, Congo red, diamine fast red, diamine blue 3-B, night blue, eosin, and Biebrich scarlet. The adsorption of some of these dyes was markedly increased by acidifying the solution.

Certain facts established regarding the adsorption of dyes by charcoal and by silicon, iron, and aluminum oxide gels have a bearing on the adsorption of dyes by soils. It was shown by Michaelis and Rona (13) that the capacities of the so-called "pure" inorganic gels for dyes

and simpler electrolytes are dependent on impurities of Cl, Na, etc., remaining in the adsorbent after purification. The adsorption consists of an exchange between the adsorbed ion and the ion of similar charge present as impurity in the adsorbent. Charcoal seems to be an exception to many adsorbents of electrolytes in that only part of its adsorption can be attributed to this exchange reaction. This conclusion that the adsorption of dye electrolytes by inorganic gels is an exchange reaction is substantiated by the work of Wood and Wooller (21) on several alumina gels, by the work of Weiser and Porter (19, p. 1830) on a chromic oxide gel, and by the work of Weiser (18) on alumina gels.

This generalization of Michaelis and Rona, that the adsorption of dyes is an exchange reaction, applies also to the adsorption by soils, of at least the basic dyes. Some years before the work of Michaelis and Rona, Ashley (3, p. 58) suggested that the adsorption of malachite green by clays was an exchange reaction "with the bases that stabilize the gel." Rohland (14, p. 381) also suggested that the adsorption of dyes by soils was often connected with exchange reactions. The data of Michaelis and Rona showed that kaolin takes up basic dyes in this way. And recently Anderson and Mattson (2) showed quantitatively that the adsorption of methylene blue by two widely different soils was an exchange reaction, at least up to a certain point. This was substantiated qualitatively by Wilkinson and Hoff (20) for several soils. The adsorption of basic dyes by soils is thus similar to the more thoroughly studied base exchange reaction with neutral salts.

If the facts established for the adsorption by soils are compared with the facts established for adsorption by artificial inorganic gels, an indication is obtained of how much acid dye soils should adsorb. Apparently the artificial gels adsorb basic dyes by exchange with cations and acid dyes by exchange with anions. Soils adsorb basic dyes by exchange with cations, and they would presumably adsorb the anions of acid dyes by exchange. Since soils seem to adsorb dye cations and the cations of simple salts to about the same extent, they would be expected to adsorb dye anions and simpler anions in equivalent amounts. Previous studies show, however, that soils adsorb only small quantities of the simple inorganic anions, if any; hence little if any adsorption of acid-dye anions would be expected. This conclusion is in harmony with the findings of certain investigators cited but is not in agreement with the data of others.

EXPERIMENTAL PROCEDURE

In the adsorption determinations, the soil was shaken with a dye solution and the unadsorbed dye was determined colorimetrically after the suspension had been clarified by centrifuging. All adsorption determinations were conducted with a 0.1 per cent dye solution which was made from a clear, 0.2 per cent stock solution. The stock solution was prepared by passing a dye solution through a Pasteur-Chamberland filter and adjusting to 0.2 per cent strength after determining the solid present in an aliquot evaporated on the steam bath. The weights of soil and volumes of dye used in different determinations were varied in such a way that about half of the dye supplied remained unadsorbed in each case; thus the adsorbed dye was in equilibrium

with an approximately 0.05 per cent solution. It was usually necessary to make several preliminary tests in order to determine the proper quantities of dye and soil to use. The soil and dye solution was shaken for 17 hours in an end-over-end shaker, and the dye remaining unadsorbed was then determined colorimetrically as follows: After the soil had settled somewhat, approximately 40 c. c. of the supernatant solution was withdrawn and centrifuged for 30 minutes, or until clear. In some cases a drop or two of dilute hydrochloric acid was added to facilitate the flocculation of clay. The color of the clarified solution was then read in a Dubosq colorimeter against that of a 0.05 per cent solution of the dye, similarly acidified when acid was used in clarification.

The soils used in this study were selected from a number of those utilized in different investigations of this laboratory. The approximate colloid content and the composition of the colloid were known in each case.

ADSORPTION OF SODIUM CARMINATE BY SOILS

The first acid dye used was sodium carminate. This, according to Way (17), is strongly adsorbed by clays. Solutions made of three different lots of sodium carminate purchased from a chemical supply house appeared to be highly but unequally adsorbed. However, a solution of one of these lots treated in a different manner was not adsorbed at all, and a fourth lot prepared from carminic acid and sodium hydrate likewise showed no adsorption at pH 7.2. Only a general description will be given of determinations made with the first lot of this dye, since there is doubt as to the reliability of the results as adsorption data.

Many determinations were made with the first stock solutions with a view to standardizing a method for comparing the adsorptions of different soils. It was found that decolorization of the dye solution was markedly affected by the time of shaking. Of the dye removed in 16 hours, about 70 per cent was taken out in the first 4 hours; but after 4 hours the rate of removal seemed to be practically constant up to 70 hours. Further, it was found that although the quantity of dye adsorbed showed some correspondence with the concentration of the dye solution, it was nearly a straight-line function of the quantity of dye supplied. These facts indicated that something besides an adsorption of an electrolyte was taking place. It was also found that duplicate determinations agreed well if made on the same day or a few days apart, but if made several weeks apart the duplicates varied widely. It then became apparent that the stock solution was deteriorating and that a precipitate was forming in the originally clear solution. Part of the precipitate was obviously due to a fungous growth, which Charles Thom of this bureau identified as a species of *Aspergillus*.

A few crystals of thymol added to a new stock solution seemed to be effective in keeping it free from fungous growth, but this solution also obviously changed on standing a few weeks.

Probably little significance should be attached to results obtained with these stock solutions since they deteriorated more or less and since subsequent lots of dye gave quite different results. However, such results as were obtained were only in very general accord with the idea that colloids with low silica-sesquioxide ratios would adsorb more of an acid dye than colloids with high silica ratios. The Sharkey soil, Norfolk subsoil, and Nipe soil, containing colloids with silica-sesquioxide ratios of 3.11, 1.60, and 0.31 removed, respectively, 0.074, 0.303, and 0.489 g. of dye per gram of colloid; but four other soils containing colloids with ratios of 1.53 to 1.70 removed quantities of dye varying between 0.075 and 0.303 g. per gram of colloid.

A new stock solution was prepared in the same manner as the first two, except that the filtered dye solution was rendered slightly acid, shaken occasionally for three days, and again passed through a Pasteur-Chamberland filter. After removal of the precipitate by filtration, the solution was neutralized with dilute NaOH and then diluted to 0.2 per cent. The addition of acid to the dye was prompted by the idea that some of the dye in the first two solutions might have been in a finely divided colloidal state which passed through the filter but later coagulated and contributed to the gradual weakening of the dye solution. It was thought that if this were the case, acid would help to coagulate and remove an unstable part of the dye from the stock solution. This solution had less color than the preceding ones similarly acidified, owing either to mode of preparation or difference in the composition of the sample, although it presumably contained the same concentration of dye. This solution was only slightly adsorbed by the Sharkey soil and the Norfolk subsoil, and not at all by Nipe clay and three other soils.

A fourth lot of dye was then prepared from some supposedly pure carminic acid purchased from another chemical supply house. Slightly less than the theoretical quantity of sodium hydroxide required to form sodium carminate was added to a solution of this carminic acid and the pH was brought to 7.2 by the addition of dilute sodium hydroxide. This dye solution was likewise not adsorbed by the Sharkey soil or the Norfolk subsoil until the pH was raised to 7.7, and then only slightly.

The failure of these soils to adsorb the acid-treated and prepared dye, except as noted, was assumed to be due to the kind of surface exposed by the colloid. It should be possible to change the nature of this surface very markedly by treatment with iron and aluminum salts. The two soils were therefore treated with ferric chloride and with aluminum chloride. Two lots of each soil were shaken for 17 hours with 5 per cent solutions of each chloride, in the proportion of 5 g. of soil to 100 c. c. of solution. The soils were then filtered and washed with distilled water. One half of each sample was washed twice, and the other half was washed repeatedly, about 15 times. After the soils had been dried at 105° C. and exposed to the air for a day or two, determinations were made of the adsorptions, as shown in Table 1.

TABLE 1.—*Adsorption of sodium carminate by soils treated with ferric and aluminum chlorides*

Soil	Treatment	Dye ad- sorbed per gram of colloid
		Gram
Norfolk fine sandy loam subsoil	FeCl ₃ , washed twice	0.248
	FeCl ₃ , washed repeatedly	.243
	AlCl ₃ , washed twice	.224
	AlCl ₃ , washed repeatedly	.219
Sharkey clay	FeCl ₃ , washed twice	.417
	FeCl ₃ , washed repeatedly	.383
	AlCl ₃ , washed twice	.300
	AlCl ₃ , washed repeatedly	.250

Evidently entrained soluble iron and aluminum chlorides played no appreciable part in the high adsorptions observed, for samples washed repeatedly removed almost as much dye as samples washed only twice. The high adsorptions may therefore be ascribed to changes in the colloids brought about by treatment with the salt solutions. These changes are not certain, but the following are to be expected: Some substitution of the exchangeable soil bases by iron and aluminum ions, and a union of the hydrous oxides or oxy-chlorides with the soil colloid particles. There is an indication in the comparative quantities of dye taken up by the two soils that removal of the dye was due chiefly to hydrous oxides precipitated on the surface of the colloids rather than to a precipitation of the dye anion by iron or aluminum ions released from the treated colloid by the sodium ions of the dye solution. If substituted iron or aluminum ions had been chiefly responsible for removal of the dye, it would seem that the Sharkey soil should have removed several times as much dye as the Norfolk, since its exchange base capacity is several times that of the Norfolk colloid (2, p. 23). As a matter of fact, the total quantity of dye removed by the four Sharkey samples is only 1.45 times the quantity removed by the four Norfolk samples. Curiously enough, the surface exposed by 1 g. of the Sharkey colloid is about 1.42 times that of the Norfolk colloid, as shown in a previous publication (2, p. 4).

There is thus some ground for assuming that treatment of the Sharkey and Norfolk soils with iron and aluminum chloride solutions introduced a reactive surface and that failure of the untreated soils to adsorb the sodium carminate to the same extent was due to lack of such a surface. The reactive surface introduced probably consisted of freshly precipitated gels of the hydrous oxides with more or less adsorbed chloride. As will be shown later, there is reason for believing that such a surface would lose reactivity on prolonged washing and subsequent aging.

ADSORPTION OF ORANGE II AND PONCEAU BY SOILS AND ARTIFICIAL GELS

A number of other acid dyes were obtained from the color laboratory of the Bureau of Chemistry and Soils. These were tested qualitatively to see if they were suitable for adsorption determinations made by the colorimetric method. Since the soil suspensions

were clarified by the addition of a drop or two of acid and subsequent centrifuging, it was necessary to use a dye that was not precipitated by small amounts of acid and one that had a constant color in the presence of different degrees of acidity. Many dyes did not meet these requirements. Solutions of Congo red and metanil yellow, for instance, were almost completely precipitated by small amounts of acid. Ponceau and orange II, however, seemed to be satisfactory for this work.

These two dyes were used in adsorption determinations with several soils containing colloidal materials with widely different silica-sesquioxide ratios. Both water and N/2,000 hydrochloric acid solutions of the dyes were used. The hydrogen-ion concentrations of the dye solutions in contact with the different soils were determined with the hydrogen electrode. This determination is probably not very reliable in the presence of dye, but the pH values obtained for the soils in the unacidified dye solutions were about the same as for the soils in distilled water. The 0.1 per cent solution of ponceau in water had a pH of 7.2. The results obtained are shown in Table 2.

TABLE 2.—*The adsorption of orange II and ponceau in water and in N/2,000 HCl by soils containing colloids with different silica-sesquioxide ratios*

Soil	Orange II adsorbed per gram of colloid—		Ponceau adsorbed per gram of colloid—		SiO ₂ Al ₂ O ₃ + Fe ₂ O ₃ ratio of colloids	pH values of ponceau and soil—	
	In water	In N/2,000 HCl	In water	In N/2,000 HCl		In water	In N/2,000 HCl
	Mgm 20 0	Mgm 33 0	Mgm 0	Mgm 0		pH 6.3	pH 5.9
Sharkey clay, A horizon.			3 0	5 0	1.88		
Iredell loam, B horizon.			0	0	1.60	5.5	4.7
Norfolk fine sandy loam, B horizon.	0	9 0	9 0	19 0	1.50		
Davidson clay loam, B ₁ horizon.			10.0	12 0	1.35	5.1	4.7
Cecil sandy loam, A ₂ horizon.	14 0	16.0	13 0	18 0	.31	7.1	7.2
Nipe clay, A horizon.	22 0	30.0					

The small adsorptions in water solutions are in strong contrast with the adsorptions obtained in previous work with the basic dye malachite green (10, p. 11). Whereas the six soils used in this work adsorbed from 0 to 22 mgm. of orange II and 0 to 13 mgm. of ponceau per gram of colloid, the 30 soil colloids used in the earlier work adsorbed from 77 to 431 mgm. of malachite green.

The small adsorptions of these acid dyes are, on the whole, related only slightly to the silica-sesquioxide ratios of the colloids. The four adsorptions of orange II show no correspondence to these ratios, and the lesser quantities of ponceau adsorbed show only a slight correspondence.

The use of N/2,000 hydrochloric acid increased the adsorption measurably; but the adsorption of these acid dyes was still small as compared with the adsorption of a basic dye. It should be noted, however, that owing to the neutralizing action of the soil, the use of this dilute acid increased the hydrogen-ion concentration of the solution only slightly.

Determinations were then made of adsorption from a much more acid solution by using N/20 hydrochloric acid solutions of the dyes. The results are given in Table 3.

TABLE 3.—*The adsorption of orange II and ponceau from N/20 hydrochloric acid solutions, and the quantities of ponceau precipitated by soluble salts extracted from the soils with N/20 acid*

Soil	Orange II adsorbed per gram of colloid	Ponceau adsorbed per gram of colloid	Ponceau precipitated by soluble salts extracted from soils with N/20 acid, cal- culated per gram of colloid
	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>
Sharkey clay, A horizon	126	20	20
Iredell loam, B horizon		223	
Norfolk fine sandy loam, B horizon	64	23	23
Davidson clay loam, B ₁ horizon		109	
Cecil sandy loam, A ₂ horizon	64	40	27
Nipe clay, A horizon	208	104	42

It will be seen that the quantities of dye removed from solution were greatly increased in all cases by the twentieth-normal acid; but it did not seem likely that the large quantities of orange II and ponceau removed represented true adsorption. It is true the results can not be attributed to precipitation of the dyes by the acid of the medium, for preliminary tests showed the dyes were not precipitated by dilute acids. Furthermore, if removal of the dyes had been due to acid precipitation, the quantities of dye removed would have been similar for all soils, and this was far from being the case. There is, on the other hand, evidence that the high adsorptions were due, in part at least, to precipitation of the dye by iron, aluminum, and other bases released from the colloidal material by the acid used.

Qualitative tests showed that small quantities of the chlorides and sulphates of iron, aluminum, calcium, magnesium, manganese, and chromium precipitated 0.1 per cent solutions of orange II and ponceau to a greater or less extent. In a quantitative test with aluminum chloride, 0.003 gm. of this salt added to 40 c. c. of 0.1 per cent dye solutions precipitated 1.5 per cent of the ponceau and 31 per cent of the orange II.

Determinations were then made to see whether the bases released from the soils by N/20 acid would precipitate the quantities of dye reported as adsorbed in Table 3. Four soils were shaken 17 hours with N/20 hydrochloric acid; the proportions of soil to acid were kept the same as in the adsorption determination, but larger quantities were used. Two portions of the clear solution from each soil, corresponding to the soil weights of the adsorption determination, were evaporated to dryness on a steam bath. One series of the evaporated soil extracts was taken up with enough acid to give a N/20 solution on addition of the dye, and the other series was taken up with distilled water. The series of extracts dissolved in acid gave no precipitates

with ponceau; but the extracts dissolved in water precipitated the quantities of dye reported in column 4 of Table 3.

The fact that the extracted salts dissolved in water did precipitate the dye, whereas the same salts dissolved in N/20 acid did not, shows that the precipitation is markedly influenced by acidity. In view of this influence of acidity, it is impossible to tell from the data given in Table 3 just how much dye was precipitated in the adsorption determinations, since the solution of extracted salts in water was obviously less acid than the solution of dye in N/20 acid used in the adsorption determination. However, the data strongly support the idea that most of the dye removed from the acid-dye solution was by precipitation with dissolved bases rather than by adsorption. This applies also to the sodium carminate in acid solution, some of which, however, was precipitated directly by the acid.

Since even soils with low silica-sesquioxide ratios adsorbed only small quantities of ponceau or orange II from water solutions, tests were conducted with artificial gels in order to see if they would take up appreciable quantities of these acid dyes. The gels used comprised the following:

(1) A commercial alumina gel that was in a hard granular condition. This gave off no water on heating to 105° C., but on ignition it lost 34.97 per cent of water as compared with the 34.57 per cent of water present in aluminum hydroxide.

(2) An air-dried alumina gel prepared in this laboratory. It contained 0.25 per cent chlorides, and lost 21.8 per cent water on drying at 105° C. and 40.35 per cent water on ignition.

(3) A commercial iron gel in a pasty condition. This lost 85.55 per cent water on drying at 110° C. and 92.85 per cent on ignition.

(4) An air-dried iron gel prepared in this laboratory. This contained 0.09 per cent chlorides and lost 3.8 per cent water on drying at 110° C. and 8.3 per cent water on ignition.

The iron and alumina gels prepared in this laboratory were made by G. J. Hough by precipitating aluminum chloride or ferric chloride with ammonia. They were washed repeatedly by the use of Pasteur-Chamberland filters and suction until the wash waters showed only traces of chlorides. Prior to use in the adsorption determinations they had been kept a few weeks in the air-dried condition. The quantities of ponceau adsorbed by these gels before and after heating are shown in Table 4.

TABLE 4.—*The adsorption of ponceau by artificial gels of the hydrous oxides of iron and aluminum*

Kind of gel	Dye adsorbed per gram of gel dried at 105° or 110° C.	Kind of gel	Dye adsorbed per gram of gel dried at 105° or 110° C.
	<i>Milligrams</i>		<i>Milligrams</i>
Commercial alumina gel.....	0	Commercial iron gel.....	228
Commercial alumina gel, dried at 110° C.....	0	Commercial iron gel, dried at 110° C..	71
Commercial alumina gel, dried at 600° C.....	0	Commercial iron gel, dried at 600° C..	14
Prepared alumina gel, air dried.....	67	Prepared iron gel, air dried.....	13
Prepared alumina gel, dried at 105° C.....	13	Prepared iron gel, dried at 110° C.....	13
Prepared alumina gel, ignited strongly by blast.....	0	Prepared iron gel, ignited strongly by blast.....	0

The failure of the commercial alumina gel to adsorb ponceau and the low adsorption of the prepared iron gel are similar to the results observed with soils. The other two gels, however, had comparatively high adsorption capacities. These results can be explained somewhat more satisfactorily than the results obtained with different soils, since previous work with the simpler, artificial gels has shown something of the characteristics of these materials affecting the adsorption of electrolytes.

It is well known that the adsorptive capacities of the hydrous oxide gels are affected by their previous history and manner of preparation. Old gels, gels that have been dried at high temperatures, and gels prepared especially free of electrolytes are likely to be poor adsorbents of electrolytes. These characteristics of age, temperature at which the gel is dried, and content of electrolytes can be used, of course, only in a general way for predicting the adsorptive capacity of a gel for electrolytes, since one condition probably influences the effect of another. It seems probable, for instance, that the loss of adsorptive capacity on aging is markedly affected by the electrolyte content, aging being more rapid when the electrolyte content is low. This is suggested by Weiser's recent work with an alumina gel free from electrolytes, prepared from amalgamated aluminum (18). This gel adsorbed alizarin when freshly prepared, but lost practically all adsorptive power in a few days.

The alumina and iron gels which adsorbed comparatively large amounts of ponceau lost the greater part of their adsorptive capacities when dried at 110° C. The failure of the commercial alumina gel to adsorb may be attributed to a possible drying at high temperature or to aging. This preparation, which was hard and granular, gave off no water at 105°, although on ignition it gave off almost the correct amount of water for crystalline aluminum hydroxide. The difference in the adsorptive capacities of the prepared alumina and iron gels may be attributed partly to a difference in purity, the alumina gel containing 0.25 per cent chloride and the iron gel 0.09 per cent. The high adsorption of the commercial iron gel is probably to be similarly explained.

These results with alumina and iron gels tend to confirm those obtained by several other investigators previously cited in showing that all extended iron and aluminous surfaces do not adsorb acid dyes. Adsorption evidently takes place only when these surfaces are in a reactive condition.

DISCUSSION OF RESULTS

The results obtained with these three acid dyes, sodium carminate (prepared from carminic acid), orange II, and ponceau, indicate that most soils have very small or almost no adsorptive capacities for acid-dye anions. Even soils containing colloids with low silica-sesquioxide ratios adsorbed very little. The comparatively high adsorptive capacity of charcoal for both acid and basic dyes, observed by Michaelis and Rona (13), suggests that possibly soils high in organic matter might adsorb more than the soils used in this investigation.

This conclusion of the low adsorbability of acid-dye anions, based on work with three acid dyes, is in harmony with the conclusions of various investigators that adsorption of the simpler anions Cl , NO_3 ,

and SO_4 by soils is negligible in neutral solutions. Some of the previous work on dye adsorption by soils also indicates a negligible adsorption of acid-dye anions; but the results of other investigators are apparently contradictory to this conclusion.

It is of course evident from the results of previous investigators that soils may in some cases take up appreciable quantities of certain acid dyes. But it is questionable whether in these cases the removal of the dye is a true adsorption of anions. The results obtained in this study and the known characteristics of dye solutions suggest that the high adsorptions previously reported may have been due to removal of dye colloids, to precipitation by acid, or to precipitation by bases in solution, rather than to adsorption.

It is well recognized that dyes, with their large molecules, tend to form colloidal solutions; some dyes, as Congo red and night blue, seem to be distinctly colloidal; others are what Freundlich (9, p. 893) terms "halbkolloide"; and some dyes, as eosin and methylene blue, are dispersed chiefly as electrolytes. A finely divided suspension of the more colloidal dyes might well be taken up by the only slightly dispersed soil colloids on prolonged shaking when anions would not be adsorbed. Michaelis and Rona (13), for instance, noted a removal of the colloidal Congo red by kaolin but no adsorption of potassium eosinate. The results obtained in this work with the first two lots of sodium carminate probably were also due in part to a removal of dye colloids. Furthermore, many acid dyes are precipitated or coagulated by slight concentrations of acid and by small quantities of the salts of calcium, magnesium, iron, aluminum, manganese, or other bases. In some cases the precipitate formed may become apparent only on centrifuging. Some high adsorptions of acid dyes previously reported might well have been precipitations of the dye with bases released from the soil colloids by base exchange with cations present in the dye preparations. The large amounts of metanil yellow that Marker and Gordon (12) report as adsorbed by silica, alumina, and iron gels were probably simple precipitations, by acid alone in the silica gel test and by acid or dissolved iron and aluminum in the tests with alumina and iron gels.

The idea that soil colloids might be better characterized by the comparative quantities of acid and basic dyes adsorbed than by the single adsorption of a basic dye or inorganic cation is apparently not well founded. The results of this study indicate that the quantity of acid dye taken up is either too small to be significant or is open to question as being true adsorption.

The somewhat negative results of this investigation are, however, suggestive of certain features of the surface exposed by soil colloids. The small adsorptions reported bear little relation to the comparative quantities of sesquioxides in the colloid and probably they afford little indication of the comparative amounts of iron and aluminum present in the surface either as oxides or silicates. A failure to adsorb simple anions and the complex acid-dye anions might, of course, be attributed to a lack of iron and aluminum radicals in the surfaces exposed to solutions; but it seems more reasonable to attribute this lack of adsorption to an unreactive condition of these basic radicals, if work on artificial iron and alumina gels is considered.

Data given in Table 4 and the results of other investigators show that some alumina and iron gels are active adsorbents, whereas others are not. The adsorptive gels seem to be those that are freshly prepared and those that contain an ionic impurity, either adsorbed or retained by the gel during its preparation. These facts might be interpreted in the case of an alumina gel, for example, by assuming that a pure, freshly prepared alumina gel contains some exchangeable hydroxyl ions, while an impure gel contains some reactive aluminum chloride, sulphate, or nitrate; that on aging of the pure gel or on removal of the anion from the impure gel, these reactive materials tend to pass into an unreactive hydrous oxide surface. The observed changes in the structure of gels that take place on aging might also be dependent to some extent on such hypothetical chemical changes.

The preceding facts and hypotheses may not apply to soil colloids, for the soil colloidal material is certainly more complex than an artificial hydrous oxide gel. But on the assumption that they do apply, the following hypotheses are suggested: Soil colloids being thoroughly aged, an adsorption of anions would not be expected unless adsorbed anions were already present. The low adsorptive capacity for acid-dye anions, therefore, indicates that the iron and aluminous surfaces carry few, if any, adsorbed anions. This may be due to such surfaces being present in an unreactive condition as highly insoluble hydrous oxides. Such surfaces might be rendered reactive to anions by solutions sufficiently acid to permit some ionization.

SUMMARY

The fact that the adsorbed cations of the basic dyes by soil colloids vary roughly with the proportion of silica to iron and aluminum in the colloid suggests that a similar relationship might obtain for the adsorption of acid-dye anions. Although adsorption of the simple anions from neutral solutions seems to be negligible, several investigators have reported appreciable adsorptions of dye anions of the acid dyes.

Data are given on the adsorption of the anions from water and weakly acid solutions of the acid dyes, sodium carminate, orange II, and ponceau. Several soils containing colloids with widely different silica-sesquioxide ratios adsorbed these dyes slightly or not at all from water solutions. Adsorptions from N/2,000 acid solutions of the dyes were larger but also inappreciable. From the N/20 acid solution large quantities of ponceau were removed by some soils; but evidence is given that removal of the dye in this case was a precipitation of the dye with bases dissolved from the soil colloids rather than a true adsorption.

It is concluded that the true adsorption of anions of the acid dyes by soil colloids is negligible, like that of the simpler anions. The comparatively large adsorptions of acid dyes reported by several earlier investigators are ascribed to the use of colloidal dyes and to precipitations of the dyes by acid or by bases released from the soil colloids.

The apparent lack of adsorptive capacity for anions is discussed in its relation to the nature of the surface exposed by soil colloids.

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THE RELATIVE RESISTANCE OF VARIETIES AND SPECIES OF CITRUS TO PYTHIACYSTIS GUMMOSIS AND OTHER BARK DISEASES¹

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INTRODUCTION

Although the great importance of knowing the response of the varieties and species of Citrus to various pathogens is at once apparent, records of complete and thoroughgoing tests and surveys are not numerous. The dearth of data acquired by artificial inoculation of the experimental hosts under more or less accurately controlled or comparable conditions is in part understandable when one considers the general feeling among growers that such inoculations may result in injury and possible death of valuable plants. Also there is the difficulty of obtaining the many varieties under uniform conditions of age, development, and health.

REVIEW OF LITERATURE

The work of Lee (5, 6²), Peltier (9), and Peltier and Frederick (10, 11) on the susceptibility and resistance of various rutaceous hosts to citrus canker is noteworthy from the standpoint of the number and diversity of plants tested and observed. In descending order of susceptibility stand pummelo, trifoliolate orange, the various limes, sweet orange and lemons, sour orange, varieties of the mandarin group, calamondins, citrons and kumquats, the last four being considered commercially resistant. Lee has found the Triumph variety of pummelo to exhibit a slight resistance to the bacterium, and he classifies also Tahiti lime as commercially resistant. McLean (7) and McLean and Lee (8) noted a correlation between the width of the stomatal slit and susceptibility to canker, their explanation being that the narrow stomata offer less easy ingress to water which may be laden with the causal organism.

Fulton (2) in his extensive investigation on the susceptibility of Citrus varieties to the West Indian lime anthracnose organism, *Gloeosporium limetticolum* Clausen, showed that only the West Indian and Dominican thornless varieties of lime are very susceptible, six other varieties of lime giving no positive indication of susceptibility either in the greenhouse or in the field. Certain strains of citron when wounded and kept under greenhouse conditions became infected to a very limited extent. All other genera, species, varieties, and hybrids of the Rutaceae tested were either immune to the disease or could not be unquestionably infected. On the other hand the anthracnose or withertip fungus, *Colletotrichum gloeosporioides* Penz., does not limit

¹ Received for publication June 2, 1930, issued September, 1930. Paper No. 222, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif. This is the third of a series of papers (3, 4) on the parasitism of *Phytophthora citrophthora* (Sm. and Sm.) Leonian.

² Reference is made by number (italic) to Literature Cited, p. 424.

its attack to a single species but parasitizes the mature twigs and fruit of many species (1, p. 287-293).

A third citrus disease on which extensive tests have been made is verrucosis or scab caused by *Sphaceloma fawcettii* Jenkins. Winston (12) and Winston, Bowman, and Bach (13) have obtained results which enabled them to separate the various rutaceous hosts into four categories based on their susceptibility, resistance, and immunity to the disease. Among the better known varieties and species, sour orange and lemon were severely attacked, the tangerine and some pummelos moderately attacked, sweet orange and Tahiti lime only rarely parasitized, while citron, kumquat, and the Royal and Triumph varieties of pummelo were not attacked. In his 1923 publication Winston (12) records that the fruit of pummelo is susceptible to scab only from its earliest formation up until it has reached three-fourths of an inch in diameter, after which it becomes immune. Similarly, leaves are immune after they reach one-half inch in width. With *Pythiacystis* rot the writers have observed a relationship approximating the opposite of that with scab, namely, in the case of twigs, branches, and leaves, the more mature the organ the greater is its susceptibility. While all ages and sizes of fruit are susceptible to *Pythiacystis* brown rot, immature fruit decays less rapidly than ripe fruit. The investigations referred to above and the data presented in this paper, the latter being a record of tests made by the writers during the years 1926, 1927, and 1928, constitute the more extensive contributions to the study of disease resistance and susceptibility in Citrus.

Records of observations on the response of the more important commercial members of the genus Citrus to bark diseases cover a period of years approximately concurrent with the commercial production of citrus fruit. As a result it is now definitely known generally that lemon, orange, and pummelo are susceptible to *Pythiacystis* gummosis and that sour orange is resistant; that the sweet orange and pummelo are susceptible to psorosis; and that the lemon stands with sour orange in resistance. To decorticosis the citrus hosts react still differently, lemon alone being susceptible, and sweet orange, pummelo, and sour orange completely resistant or immune. Since with *Pythiacystis* gummosis a good indication of the reaction of a given species or variety of Citrus may usually be obtained in 40 days, whereas with psorosis and decorticosis several years are required even on the more susceptible hosts, the experimental data reported here are confined to the first disease. For purposes of comparison it may be recalled from previous discussion that sour orange, lemon, and pummelo are susceptible to scab, while the sweet orange is commercially resistant. All four species, however, are found to be susceptible to canker. These comparisons of the very variable reactions of the four important commercial species of Citrus indicate that we should look to the specific biochemical make-up of the protoplasm for hypotheses that may lead to explanation.

MATERIALS AND METHODS

The inoculation tests on brown-rot gummosis reported here were made in the variety orchards of the University of California Citrus Experiment Station. Subcultures of a strain of *Phytophthora citrophthora* (Sm. and Sm.) Leonian, known as 1309-a, which was isolated from a diseased lemon tree at La Habra, Calif., were used throughout

the work. The surfaces of the places on the tree trunk to be inoculated were in all cases first rubbed with a towel dipped in 95 per cent alcohol. The 1926 inoculations were confined to 9-year-old trees of the Rubidoux plot, and were made during the period January 2 to 5. A small piece of mycelium from a 6-day-old glucose-potato agar slant culture was inserted under the flap of bark made by a small vertical slit. The inoculations were made on the east or southeast side of the trunk and on one main limb and were covered with pieces of oiled paper, the edges of which were glued. The 1927 inoculations were made during the period February 22 to March 4 in fields 1 and 12. Each tree received one inoculation on the west side of its trunk in the manner described.

Inoculations in 1928 were made during March 15 to March 26 in all three of the above-mentioned groves. In general, depending upon the size of the tree, four inoculations, two about 6 inches and two about a foot from the ground, were made on the trunk of each tree, the fungus being inserted at each of the four points of the compass. Instead of a vertical slit a small cylindrical hole, 4 mm. in diameter, was drilled through the bark to the cambium with a flamed cork borer. The inoculum was placed directly on the cambium and the hole sealed with a piece of adhesive tape, the portion directly over the inoculum being lined with a half-inch square piece of paper to prevent fungicidal action by the zinc oxide of the tape.

There were three sets of observations and measurements. At the first inspection, 40 to 45 days after inoculation, only external length and breadth of the lesion were measured, the disease being allowed to run its course undisturbed until 20 days later when similar external measurements were made. The canker was then excised, and measurements were taken of the lesion as it appeared on the surface of the cambium and wood. Finally the area of the lesion on the excised bark was measured by means of a planimeter.

TABLE 1.—Size of lesions in square centimeters made and amount of gum formed on 11-year-old citrus trees by *Phytophthora citrophthora* in 60 days

[Field No. 1 Inoculations made March 22, 1928; excisions made May 22, 1928]

Species and variety	Experiment station No.	Lesion measurements						Order of resistance	Amount of gum exuding*
		North	South	East	West	Individual average	Group average		
Lemon (<i>Citrus limona</i>).									
Seedling	612			28.4	21.1	24.8	35.3	1	
Villafranca	568			33.3	21.9	27.6		2	
Do	280			29.0	38.7	33.8		3	
Kennedy	244			36.0	36.6	36.3		4	
Villafranca	390			29.2	50.6	39.9		5	
Eureka	583			56.75	25.21	41.0	6.82	6	
Belair	566			26.60	60.6	43.6		7	5
Rough	400	7.55	4.06	12.26	3.42	6.82			3
Lime (<i>Citrus aurantifolia</i>).									
Tahiti	391	1.935	1.55	3.875		1.29	4.02	1	1
Sweet (lemon), Florida									
Ida	569	3.36	4.06			3.71		2	1
Kusia	452	3.226	5.8	3.42		4.15		3	1
Sweet	654	4.52	4.06	4.06		4.2		4	2
Do	654	5.48	3.875			4.68	1.54	5	3
Rangpur	131	8.20	4.13	5.16	6.9	6.1		6	2
Citron (<i>Citrus medica</i>).									
Citron of commerce	649			1.78	1.162	1.47	1.54	1	1
Indian	138			1.613	1.613	1.613		2	1

See footnotes at end of table.

TABLE 1.—Size of lesions in square centimeters made and amount of gum formed on 11-year-old citrus trees by *Phytophthora citrophthora* in 60 days—Continued

[Field No. 1. Inoculations made March 22, 1928, excisions made May 22, 1928]

Species and variety	Experiment station No	Lesion measurements					Order of resistance	Amount of gum exuding *
		North	South	East	West	Individual average		
Pummelo (<i>Citrus maxima</i>)								
Siamese	640	2 39	1 29	1.613	2.32	1.903	1	1
Round Hawaiian	448	1 162	1 935	3.87	2.32	2.32	2	1
Imperial	596	3 74	5 94	3.42	6.58	4.92	3	2
Windsor	350	4 0	6.452	4.91	5.16	5.13	4	3
Marsh	126	4 52	6 13	6 13	4.96	5.43	5	3
Pernambuco	298	1.484	6 65	5.81	8.52	5.62	6	2
Chinese	325	6 58	4.77	5.55	5.63	5.63	7	1
Hall Silver	256	8 39	5.8	7.81	4.26	6.56	8	3
Duncan (path of infection at 45° angle)	246	6 452	11 86	8 26	21.93	12 13	9	5
McCarty	265	26 5	18 25	7 87	9 04	15 4	10	3
Sweet orange (<i>Citrus sinensis</i>)								
Navel	397	3 42	4 0	3 61	4 06	3.77	1	1
Indian River	355	4 06	4 52	4 26	3 68	4.13	2	1
Cutlin	429	5 29	3 68	4 84	5 62	4.85	3	1
Boone Early	260	4 71	2 71	6 452	5 68	4.89	4	1
Weldon	430	3.23	6 71	7 43	4 71	5.52	5	1
Hart Tardiff	570	5.62	4 84	7 29	6 65	6 1	6	2
Lue Gim Gong	255	7 74	5 16	5.36	6 452	6 18	7	2
Parson Brown	295		7 94	4 45	7.68	6 69	8	1
Madam Vinous	234	7 94	8 20	4.91	6 77	6 95	9	1
Selecta	37782	9 68	8 71	6 00	6 452	7 71	10	1
Sweet Seville	286	8 20	11 42	12.90	8.13	10 16	11	3
Mandarin (<i>Citrus nobilis</i>)								
Satsuma	592	3226	1 033	903		753	1	±
Cleopatra	270	1.484	1 42	1 42	1 613	1 484	2	1
Willow Leaf	595	4 71	4 0	4.65	2 49	3.96	3	0
Oneco	263	5 62	3 94	5 03	4 90	4 87	4	1
Clementine	279	4 38	5 49	7.75	7 23	6.21	5	1
Dancy	593	8.13	7 94	8 9	5 62	7 65	6	2
Sour Orange (<i>Citrus aurantium</i>)								
Seedling				774	6452	71	1	±
Do				1 42	9675	1.194	2	±
Paraguay	600	1 68	1 74	1.68		1 7	3	±
Standard Seedling	628	1 68	2 13	2 066	1 613	1 87	4	±
African	64	(v)	(v)	(v)	(v)	(v)	5	±
Kumquat (<i>Fortunella</i> spp.)								
Marmumi	132	.904	1 007		1 355	1 119	1	1
Nagami	268	1.29				1 29	2	1
Calamondin (<i>Citrus mitis</i>)								
Calamondin	643			1.806	1 42	1.613	1	1
Do	643	3.36		.6452		2 00	2	1
Other species								
Citrus ichangensis	1215	2 58	4 71	3.1	2 26	3.16	1	3
Do	1215	7.93		2.26	2.00	4.06	2	3
Citrus hystrix	432			.968	1 29	1 129	1	1
Do	432	2.26		2.45	1.68	2.13	2	1
Citrus limetta	758	(A)		(A)			±	
Citrus webberii	767			6.71	5.16	3.61	1	4
Do	767			54 24	26 65	40.4	2	5
Hybrids								
Tangelo (Tangerine × Pummelo)								
Sampson	399	1 613	1.613	1.613	2.77	1.902	1	±
Citrange (Trifoliate × Sweet)								
Cunningham	271	.6452	4.84	.1935	2.49	2 02	1	1
Sanford	276	2.9	4 0	.516		2.47	2	1
Savage	275	3.35	2.84	3.29		3.16	3	1
Morton	296	2.77	24.9	4.52	8.38	10.1	4	1
Rusk	301	6.20	46.25	18.25		23.57	5	5
Eatonja	309	8.39	78.07		19.0	35.15	6	5
Colman	280	(v)	(v)	(v)	(v)	(v)	7	5

* Expressed on a numerical basis, 5 representing a copious secretion, 4 medium plus, 3 medium, 2 medium minus, 1 slight, and 0 none.

† Southeast.

‡ Northeast.

§ Northwest.

|| Southwest.

† Inoculation made May 4, 1928, excision made June 11, 1928; 38 days.

‡ No involvement. Immune.

§ Slight formation of gum.

|| Girdled. Very severe. Could not excise and save bark.

TABLE 2. Average areas of lesions in square centimeters made on citrus trees by *Phytophthora citrophthora* and amount of gum exuding

Species and variety	Average of lesion measurements after						Grand average for the variety	Grand average for the species	Amount of gum exuding ^a	Average amount of gum exuding
	67 days on 9-year trees, Rubidoux plot, 1926	110 days on 2½ to 3 year trees, field No. 12, 1927	74 days on 10-year trees, field No. 1, 1927	43 days on 3½ to 4 year trees, field No. 12, 1928	70 days on 11-year trees, Rubidoux plot, 1928	60 days on 11-year trees, field No. 1, 1928				
Lemon (<i>Citrus limonia</i>)										
Ponderosa	—	—	16.25	—	—	—	16.25	—	—	+4.0
Villafraanca	42.0	—	60.00	—	—	33.8	45.3	—	—	+4.8
Belair	—	—	—	—	—	43.6	43.6	—	—	+7.0
Eureka	40.25	—	58.5	—	—	41.0	46.58	53.76	—	+4.4
Seedling	—	—	85.6	—	—	24.8	55.2	—	—	+4.5
Lisbon	29.1	—	125.0	—	—	—	77.0	—	—	+5.0
Kennedy	—	—	148.5	—	—	36.3	92.4	—	—	+5.0
Rough	11.0	13.12	23.87	4.64	3.9	6.82	10.56	10.56	+1.33	84.67
Lime (<i>Citrus auranti-folia</i>)										
Bearse Seedless	(c)	(c)	(c)	(c)	(c)	(c)	(c)	(c)	(c)	(c)
Kusia	—	—	9.0	—	—	4.15	6.57	—	—	+1.0
Sweet (lemon)	—	—	10.0	—	9.03	3.71	7.58	12.97	—	+2.33
Sweet	—	—	9.0	—	6.24	4.44	6.56	—	—	+2.5
Tahiti	17.5	—	13.5	—	—	1.29	10.76	—	—	+2.0
Rangpur	22.5	—	38.5	—	—	6.1	22.37	—	—	+2.66
Persian Sweet	24.0	—	—	—	—	—	24.0	—	—	+5.0
Citron (<i>Citrus medica</i>)										
Italian	(d)	(d)	(d)	—	—	1.613	1.613	5.701	—	+1.0
Indian	—	—	—	—	—	—	7.25	—	—	+1.0
Chinese	—	—	7.25	—	—	—	7.25	—	—	+2.0
Citron of Commerce	5.25	—	18.0	—	—	1.47	8.24	—	—	+1.66
Pummelo (<i>Citrus maxima</i>)										
Shanese	—	—	—	—	—	1.903	1.903	—	—	+1.0
P. J. Wester seedling	—	—	3.75	—	—	—	3.75	—	—	0.0
Imperial	—	—	—	—	—	4.92	4.92	—	—	+3.0
Windsor	—	—	—	—	—	5.13	5.13	—	—	+3.0
Red Fleshed	—	—	—	—	7.37	—	7.37	—	—	+3.5
Marsh	18.0	—	8.00	—	6.83	5.43	9.56	10.54	—	+3.0
Triumph	4.5	—	13.5	—	7.64	—	8.55	—	—	+4.0
Hall Silver Cluster	13.5	—	8.75	—	6.29	6.56	8.78	—	—	+3.0
Florida seedling	10.0	—	—	—	—	—	10.0	—	—	+2.0
McCarty	—	—	—	—	4.97	15.4	10.18	—	—	+3.5
Duncan	12.0	—	—	—	7.162	12.13	10.43	—	—	+4.0
Round Hawaiian	—	—	25.0	—	4.805	2.32	10.71	—	—	+2.3
Chinese	—	—	25.5	—	—	5.03	15.56	—	—	+1.0
Pernambuco	5.25	—	—	—	32.53	5.62	14.47	—	—	+3.3
Alonso	17.5	—	—	—	—	—	17.5	—	—	+3.0
Royal	8.0	—	—	—	51.7	—	29.85	—	—	+4.0
Sweet orange (<i>Citrus sinensis</i>)										
Golden Buckeye	(c)	(c)	(c)	—	—	—	—	—	—	+3.0
Navel	—	—	—	—	—	—	—	—	—	+1.3
Indian River	3.0	—	—	—	3.645	4.13	3.59	—	—	+3.0
Jaffa	4.5	—	—	—	—	—	4.5	—	—	+1.0
Boone Early	—	—	—	—	5.34	4.89	5.11	—	—	+1.0
Brazilian	5.0	—	—	—	—	—	5.0	—	—	+1.0
Lue Him Gong	4.5	—	8.0	—	—	6.18	6.23	—	—	+2.0
Enterprise Seedless	—	—	—	—	6.56	—	6.56	—	—	+2.0
Valencia	13.1	4.8	—	2.88	—	—	6.93	—	—	+1.0
Sweet Seville	8.0	—	—	—	3.68	10.16	7.28	8.08	—	+1.3
Dugut	8.0	—	—	—	—	—	8.0	—	—	+1.0
Parson Brown	—	—	10.0	—	—	6.69	8.34	—	—	+1.0
Pineapple	—	—	9.0	—	9.28	—	9.14	—	—	+2.0
Homosassa	—	—	10.0	—	8.84	—	9.42	—	—	+1.5
Ruby Blood	10.0	—	—	—	—	—	10.0	—	—	+1.0
Navel	7.0	20.56	38.0	5.667	12.34	3.77	14.55	—	—	+2.0
Mediterranean Sweet	12.0	—	21.0	—	—	—	16.5	—	—	+2.5

^a Expressed on a numerical basis, 5 representing a copious secretion, 4 medium plus, 3 medium, 2 medium minus, and 1 slight.

^b Rough omitted.

^c Slight brown stain. Healed.

^d Flap only killed. Healed.

^e Very slight spread. Callus.

TABLE 2.—Average areas of lesions in square centimeters made on citrus trees by *Phytophthora citrophthora* and amount of gum exuding—Continued

Species and variety	Average of lesion measurements after—						Grand average for the variety	Grand average for the species	Amount of gum exuding*	Average amount of gum exuding
	67 days on 9-year trees, Rubidoux plot, 1926	110 days on 2½ to 3 year trees, field No. 12, 1927	74 days on 10-year trees, field No. 1, 1927	43 days on 3¼ to 4 year trees, field No. 12, 1928	70 days on 11-year trees, Rubidoux plot, 1928	60 days on 11-year trees, field No. 1, 1928				
Mandarin, (<i>Citrus nobilis</i>).										
Satsuma	1 125		5 25			1 04	2.47	7.634	+ .5	1.07
Willow Leaf						3 96	3.96		0 0	
Cleopatra	6 0		6 0			1 484	4.495		+ .5	
Trimble			5 0				5.0		+3.0	
Parson Special	8 0		2.5				5.25		0 0	
Oneco			7 0		5 75	4.87	5.87		+1.0	
Clementine	10 0		7 0		7 06	6.21	7 72		+1.25	
Weshart			8 0				8 0		+3 0	
King	15 0		6 0				0 5		+1.0	
Dancy	33 0		8 8		4 935	7 65	13 6		+1.5	
Lester	40 0		5 44		5 89		17.11		0 0	
Sour Orange (<i>Citrus aurantium</i>)										
Stock				1.161			1.161	4.52	+1 0	.75
Parguay			3 9			1 7	2 8		+0.5	
Seedling			5 0			1 25	3.12		0 0	
Standard			7.5		2 55		5.02		+1.0	
Florida	7 0						7 0		+1.6	
South African	8 0						8 0		+1 0	
Trifoliolate (<i>Poncirus trifoliata</i>)										
Trifoliolate Stock No 12							0.0		+1 0	2 3
Do				.355			.355	.355	+1 0	
Trifoliolate Rubidoux (Identity Questionable)					.356 25		.356 25	.356 25	+5.0	
Citrus limetta									±	
Microcitrus, (<i>Microcitrus virgata</i>)		2 7		1 27			1 99	1 99	+1 0	
Citrus hystrix			3 0			1 63	2.31	2.31	+1.0	
Citrus webberii			16.0			22.78	19.39	19.39	+4.0	
Citrus ichangensis						3.61	3.61	3.61	+3.0	
Calamondin, (<i>Citrus mitis</i>)			3 0			1 806	2.403	2 403	+1.0	
Severinia, (<i>Severinia buxifolia</i>)				387			387	.387	+2.0	
Kumquat (<i>Fortunella</i>)										1 0
Marmulini						1.119	1.119		+1.0	
Nagami						1 29	1.29	1 205	+1 0	
Tangelo, (<i>Tangerine</i> × <i>Pummelo</i>), Sampson	12 0		2 28		3 824	1.902	5 0	5.0	+1 0	
Citrange (<i>Trifoliolate</i> × <i>Sweet</i>)										
Sanford			5 0		1.13	2.47	2.87	47.72	+ .5	
Cunningham			12.5		.97	2.04	5.17		+ .75	
Savage			36.0			3.16	19.58		+2.0	
Rusk	44.0	4.52	7 0	1 51	40.52	23.57	20.19		+2.4	
Morton	22.0		82.8	2.06	5.29	10 1	24.45		+2.3	
Eatonia			42.0		6.26	35.15	27.80		+3.25	
Colman			234.0				234.0		+3.3	
Shaddock × St. Michael			3 0				3.0	3.0	+4.0	
Orange × Lemon			26.25				26.25	26.25	+3.0	
Citrangequat (<i>Citrange</i> × <i>Kumquat</i>)		2.87		.1548			1 51	1 51	+1.0	2.07
Citrumelo (<i>Trifoliolate</i> × <i>Pummelo</i>)				1.017			1.017	1.017	+2.5	
Citrangor (<i>Citrange</i> × <i>Sweet</i>)				2.192			2.192	2.192	±	
Limequat (<i>Lime</i> × <i>Kumquat</i>)		3.6		.605			2.102	2.102	+2.0	
Calamelo (<i>Calamondin</i> × <i>Pummelo</i>)		21.0		.968			10.984	10 984	+3.0	
Citruvel (<i>Navel</i> × <i>Trifoliolate</i>)				.613			.613	.613	±	
Cicitrangle (<i>Trifoliolate</i> × <i>Citrange</i>)				1.162			1.162	1.162	+1.0	

* Expressed on a numerical basis, 5 representing a copious secretion, 4 medium plus, 3 medium, 2 medium minus, and 1 slight.

DISCUSSION OF RESULTS

Table 1 gives in detail the planimeter measurements of lesions obtained in 1928 in field 1, which contain the most representative and comparable lot of trees. The various species and varieties are grouped for purposes of comparison. Table 2 is a general summary of all the inoculation data of the three years, all the lesions on all the individuals of a variety being averaged. The data shown in the second, third, and fourth columns of Table 2, which give the results of the 1926 and 1927 investigations, were not obtained by planimeter measurements but by multiplying the length of the lesion by its breadth, inasmuch as the diseased areas were irregular in shape this method of calculation yielded values from 25 to 50 per cent greater than the actual values. However, the values of the respective groups thus estimated are reasonably comparable and are therefore included.

Since the trees in field 12 were only 3 to 4 years of age and since the writers have found that the younger organs of a lemon tree are less susceptible to *Pythiacystis gummosis* than the older, the results of the inoculations in that plot are not comparable with those obtained in the Rubidoux plot and in field 1 on trees 10 years old. The group results from the Rubidoux plot and field 1 approximated each other, and for simplicity there are presented in detail only the 1928 results in field 1, as recorded in Table 1. The relation of different groups in the other orchards was found in general to be the same.

A number of important conclusions appear to be justified by the data obtained. The varieties of lemon are uniformly very susceptible, only the seedling tree giving an indication of lessened susceptibility, and suggesting but a very remote possibility of obtaining by selection a variety of lemon resistant to the parasite. The long-observed and acclaimed resistance of sour orange has been substantiated. Here again the seedling form of the variety shows a greater resistance than the budded, in fact so great a resistance that for all practical purposes it may be considered to be immune. However, one specimen of the African variety of sour orange showed no involvement of tissue, which also suggests a true immunity.

Considering further the commercial varieties the writers found that grapefruit and sweet orange were about equal in susceptibility. As a group the varieties of *Citrus marima* exhibit great variability of response to the parasite, ranging from a resistance only slightly less than that of the standard sour orange in the case of the Siamese variety to a susceptibility approaching lemon in the McCarty variety. In fact, the pummelo variety Royal Bahaman in the Rubidoux plot exceeded the most susceptible lemon as shown by an average involvement of 51.7 sq. cm. A peculiarity noted in the McCarty, the Royal, the Pernambuco, and in several other varieties of pummelo was that the disease progressed approximately at a 45° angle to the vertical. For this reason infection in these species presents a very serious aspect, that of girdling the tree possibly in a single season.

The variability in the varieties of sweet orange was less than in pummelo, and none approached the susceptibility of lemon. The average of the mandarin group was much below that of pummelo and sweet orange, being similar in this respect to rough lemon and the limes, *Citrus aurantifolia*. The Willow Leaf and Cleopatra varieties of mandarin showed considerable resistance in these tests.

According to the writers' observations one specimen of Tahiti lime could be classed with sour orange. Although general observations are that citron is very susceptible to gummosis, the two varieties inoculated here showed pronounced resistance to the strain of fungus employed. However, these two specimens of citron were apparently in a poor, nonsucculent condition, which may account for their lack of susceptibility. The evidence appears sufficient to warrant the trial of some of the unusual varieties as rootstocks, such as calamondin, and some of the hybrids—tangelo and the Cunningham, Sanford, and Savage citranges. Specimens of the Rusk citrange were very susceptible, as were also the Colman and Eatonia. The genus *Fortunella*, the kumquats, exhibited great resistance to the fungus, but because of their dwarflike growth their use as rootstocks is probably out of consideration. *C. limetta* and *C. hystrix* were resistant, *C. ichangensis* was less so, while one specimen of *C. webberii* succumbed as readily as the lemon varieties.

The amount of gum formed was approximately directly proportional to the extent of involvement of the disease. Gumming is the most prominent symptom of this disease, and on individuals of the several commercial kinds of Citrus one may obtain an approximate indication of the severity and extent of the malady by observation of the amount of gum formed.

The great variability in response to disease among the varieties of the same species and even in several areas of the trunk of the same individual suggest the desirability of a uniformly dependable biochemical test for the measurement of disease resistance. The senior author (4) has already indicated the possibility of using the degree of inhibition of the fungal enzymes by the bark of the several hosts as a test for disease resistance. It was reported that the bark of sour orange and of tangelo has consistently shown greater inhibition of the diastase of *Phytophthora citrophthora* of *Phomopsis californica* Fawcett, of saliva and malt than has that of lemon. Invertase of *Phytophthora citrophthora* similarly was inhibited more by sour orange than by lemon bark. Commercial invertase, however, was inhibited more by lemon than by sour orange. In all laboratory tests made subsequently lemon and sour orange have maintained their relative effects. Calamondin, however, which proved resistant in field tests, was about midway between sour orange and lemon in its degree of inhibition of fungous diastase. Another apparent failure of the parallelism was noted in a series in which the bark of a pummelo showed about the same inhibition of ptyalin as that of sour orange, but it should be recalled that some of the pummelos show resistance to the disease similar to that of sour orange. In general the laboratory tests approximated field observations; for example, one of the resistant citranges showed only slightly less effect on the fungous diastase than sour orange. Specimens of trifoliate orange, *Poncirus trifoliata*, in the Rubidoux plot, a species which has been used extensively as a stock in Japan and the Gulf States, proved more susceptible than lemon to the artificial inoculations, and in the laboratory less inhibitive than lemon.

While the results of laboratory tests with these carbohydrases taken as a whole are not striking they seem suggestive. It is not to be expected that all enzymes would be similarly affected. In

fact the enzyme emulsin was inhibited by sour orange only slightly more than by lemon, and urease action was stimulated by both. Likewise the pectinase of *Phomopsis californica* was inhibited only slightly and about equally by sour orange and lemon bark. This suggests that the failure of an organism to establish itself and progress in the tissues of its host may depend upon the destruction of or considerable inhibition of not necessarily all enzymes, but of one or more of its important enzymes by some cellular product of the host. Possibly the enzymes responsible for making carbon available are the important ones depressed or put out of action. In the case of a resistant host, such as sour orange, the paralyzing power may be thought of as being so great that the hyphae, handicapped by an insufficient carbon source due to the inhibition of their carbohydrases or other enzymes, can not progress so rapidly as the host callus forms; whereas in the susceptible host, as lemon, it may be postulated that there is a sufficient decrease in this antienzymic action to permit the hyphae to obtain food sufficiently rapidly for successful parasitism.

Application of the method to the proteases is the next step that suggests itself. The make-up of a species or variety is determined by the physical and chemical specificity of its protoplasm, and that in turn probably to a large extent by its own distinct proteinaceous materials. To account for the innumerable species and varieties one naturally turns to the chemistry of proteins. Of all biological substances the proteins only are sufficiently numerous to explain the specificity of such infinite variety. The infinite number of distinct proteins possible from combinations of 20 amino acids is strong support for the hypothesis that immunological specificity and probably most other features of biological specificity, as in heredity, must depend on these substances. The degree of disease production and establishment of a host-parasite relationship may depend largely on the ability of the microorganism to attack the food substances of the host, particularly the proteins.

While the extensive work reported here essentially substantiates the casual observations of many years, there are several apparent anomalies. These deviations from the expected show the necessity for making such field tests thorough and extensive. Before a species or variety is adopted commercially the response of a large number of individuals to the various pathogens should be determined under each of the several possible sets of commercial conditions.

SUMMARY

A review of the literature is given, summarizing the range of susceptibility or resistance of the various species and varieties of Citrus and Citrus relatives to canker, West Indian lime anthracnose, withertip disease, scab, psorosis, decorticosis, and *Pythiacystis gummosis*.

The inoculation tests show that dependable comparisons of the response of species and varieties of Citrus to *Pythiacystis gummosis* can be made only on trees which are all of the same age and which are under similar environmental conditions.

Great variations in degree of susceptibility were shown by different varieties of the same species, individuals of the same variety, and

even by two or more locations on the same tree trunk, necessitating several inoculations on each tree to obtain a dependable average.

The varieties of the pummelo and citrange groups showed the largest differences in response to the disease, ranging from a susceptibility exceeding that in the lemon varieties to a resistance greater than the average of the sour orange group.

Under the conditions of the tests reported here the varieties of the individual groups as a whole rank in descending order of susceptibility as follows: Lemon, citrange, lime, pummelo and sweet orange, mandarin, rough lemon, tangelo, sour orange, and kumquat.

The amount of gum formed is approximately proportional to the severity and extent of the disease.

Calamondin (*Citrus mitis*), *C. hystrix*, *C. limetta*, *C. ichangensis*, Sampson tangelo, and the Cunningham, Sanford, and Savage varieties of citrange exhibited sufficient resistance to justify their trial as possible rootstocks.

The great variability found and the difficulties experienced in obtaining uniform trees and conditions for field tests suggest the desirability of dependable biochemical tests for resistance. Determination of the degree of inhibition of fungal enzymes by substances in the bark of the several hosts may be a practical laboratory procedure. Preliminary results of such biochemical tests and a comparison with field results are given.

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THE NEMIC FAUNA OF THE SLIME FLUX OF THE CAROLINA POPLAR¹

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INTRODUCTION

In Europe the slime flux of various trees is known to harbor certain nemas. The present material, from Shepherdstown, W. Va., is derived from Carolina poplar, *Populus eugenei* Simon-Louis, which is said to suffer badly there from slime flux.

Slime flux is attributed to several agents such as fungi, bacteria, frost, etc. (Ludwig, 4, p. 81, 89, 92; Neger, 9, p. 111-113; Tubeuf, 11, p. 142-144)³ Apparently nemas have never yet been considered causal agents, and it seems rather improbable that they should be such. On the other hand, there is the fact that some of these nemic forms have never yet been found elsewhere. However, it is not certain that they are found only in slime flux and have thus to be considered as constant associates. Their modes of distribution are unknown, but it has been suggested that insects that feed on slime flux or are otherwise associated with it may play a rôle in their dispersion. (Ludwig, 4, p. 89, and Aubertot, 1, p. 334.) Of course, by such means the whole life association of slime flux might be translocated.

In Europe, *Anguillula dryophila* De Man and *A. ludwigi* De Man were found in the slime flux of the white oak, and *Diplogasteroides spengelii* De Man (5), *Mononchus muscorum* (Duj.) Bast., *Dorylaimus silvestris* De Man, *D. oxycephalus* De Man, *D. macrodorus* De Man, and *D. spengelii* De Man in slime flux of the horsechestnut. The former two are closely related to *Anguillula aceti* (Muell.) Muell. found in vinegar, *A. silusiae* De Man in beer (1), and *A. nepenthicola* Menzel in cans of *Nepenthes gymnamphora* Nees from Java (8). *Diplogasteroides spengelii* was the first known species of its genus; two others were later described by Micoletzky, namely, *D. africanus* Micoletzky from fresh water in South Africa (6) and *D. variabilis* Micoletzky from soil soaked with fresh water near Czernowitz (Cernauti), in Bukowina, Rumania (7). A fourth, *D. stigmatus*, n. sp., occurs in the present material. It is quite a distinct species belonging to the group with paired ovaries, that is, with *D. variabilis* and *D. africanus*; *D. spengelii* has only a single ovary. The present slime flux of poplar contains another apparently new species, a *Diplogaster* that may be named *D. americanus*, n. sp.

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² The writer is indebted to Howard H. Somerville, of Shepherdstown, W. Va., for the material used in this study, which reports nemie fauna in slime flux for the first time in the United States

³ Reference is made by number (italic) to Literature Cited, p. 433.

Both of these new species were found in larval as well as adult form and undoubtedly pass their whole life cycle in the flux. *Diplogasteroides stigmatus*, however, outnumbered *Diplogaster americanus* as follows:

Diplogasteroides stigmatus.—Larvae, 203; females, 178; males, 127; total number, 508; sexual number, 71.4.

Diplogaster americanus.—Larvae, 15; females, 18; males, 8; total number, 41; sexual number, 44.4.

The nature of the food of the two species found in the present material is not known. It is thought, however, that some other microorganisms (fungi or bacteria) usually present in slime flux may be ingested.

CLASSIFICATION OF THE DIPLOGASTERIDAE

In a previous paper (10) it was pointed out that *Diplogasteroides* is considered to be a good genus, contrary to Baylis and Daubney (2). The *Diplogaster* found in the poplar slime flux belongs to that group of this genus in which a wide anterior portion of the pharynx is followed by a less wide, but not narrow, cylindrical or somewhat conical posterior one; the longitudinally striated anterior portion has a large dorsal or subdorsal onchium. The group contains also *Diplogaster striatus* Bütschli, *D. liratus* A. Schneider, *D. trichuris* Cobb, and others. In the previous paper (10) it was pointed out that the present writer fully agrees with Cobb's view (3) that the genus *Diplogaster*, as conceived to-day, includes forms belonging to at least three different groups. An up-to-date revision of this genus might well split it into a number of genera coordinated with *Diplogasteroides*, *Neodiplogaster*, *Rhabditolaimus*, and others. All these are members of the family *Diplogasteridae*, a family well characterized by the shape of its esophagus and of the male sexual apparatus. The family is interesting because of the variety of ecological conditions under which its members live and also because of the relationship to the *Rhabditidae*, mononchs, and others. Ecologically, the *Diplogasteridae* have free-living, saprophytic, saprozoic, plant-parasitic, and animal-parasitic members. It is undoubtedly a group branching out in many directions.

DESCRIPTIONS OF NEW SPECIES

DIPLOGASTEROIDES STIGMATUS, N. SP.

The form of *Diplogasteroides stigmatus* (fig. 1) resembles *D. spengelii* from Europe but may easily be distinguished because the female apparatus is amphidelphic, whereas that of *D. spengelii* is prodelphic. There are a number of other differences, but this one is perhaps the most outstanding. *D. variabilis*, which is also amphidelphic, can at once be distinguished because of the large amphid which is in the latitude of the proximal end of the pharynx. The same is the case with *D. africanus*, which in addition has two ringlike structures at the base of the pharynx.

The head end of *Diplogasteroides stigmatus* is convex-conoid in both sexes, the tail end much elongated and filiform. The cuticle is annulated very finely, but with high magnification this annulation is resolved into transverse series of dots. These, however, are not quite uniform, being larger and more prominent on the lateral body surface, especially in the anal region.

Thus the cuticle of the present species differs from that of the European species, *Diplogasteroides spengelii*, which has longitudinal striae and no special markings on the lateral surface. *D. variabilis* also has longitudinal striae and in addition a lateral wing; and the same striae are found on *D. africanus*, but here the wings are absent. The head end has a single circle of papillae. In *D. spengelii* there is an additional circle of 12 papillalike structures nearer the oral opening;

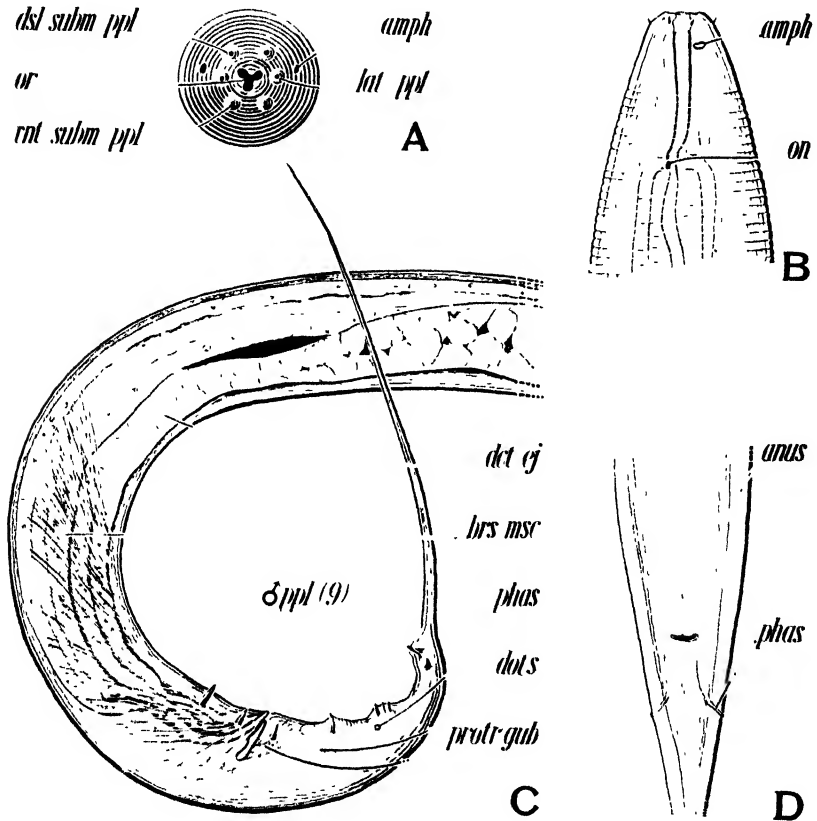


FIGURE 1.—*Diplogasteroides stigmatus*, n. sp.

- A.—Front view of head. *amph*, Amphid; *dsl subm ppt*, dorso-submedial papillae; *lat ppt*, lateral papillae; *or*, oral opening; *rnt subm ppt*, ventrosubmedial papilla. \times about 1,433
 B.—Head end in side view. *amph*, Amphid; *on*, onchium. \times about 1,090
 C.—Tail end of male in side view. *hrs msc*, Bursal muscles; *det ej*, ductus ejaculatorius; *dots*, dots of the cuticle as they appear along the lateral line; *det ppt* (9), the nine copulatory papillae of the male; *phas*, phasmid; *protr gub*, protractor gubernaculi. \times about 533
 D.—Ventral view of anal region of female. *anus*, Anus; *phas*, phasnid. \times about 533

but De Man says their true nature is uncertain. In the present species no such structures were seen. The amphids of *D. spengelii* are located about opposite the middle of the pharynx; in the present species, however, they are farther forward, almost at the end of the first fourth of the pharynx. The latter is narrower but longer than in the European species. Its walls seem not so strongly cuticularized. A minute toothlike structure was seen at the base of the

cylindrical portion of the pharynx. The esophagus and intestine do not show any variations from the European species, nor do the nerve ring and the excretory pore. The female genital apparatus is amphidelphic. Only one to two eggs are usually seen in each uterus; segmentation has begun when they are deposited. The ovaries are reflexed. The single testis of the male also is reflexed. The spicula are rather slender, arcuate, and cephalated at the proximal ends. The gubernaculum is very different from that of *D. spengelii*; it is longer and more like a spiculum. The arrangement of the muscles of the spicular apparatus may be seen in Figure 1, C. The oblique copulatory muscles are strongly developed and extend about three to four times the length of the spicula in front of the anus. The arrangement of the male copulatory papillae is also different from that of any other Diplogasteroides. A comparison (fig. 1, C) shows best the manner of their arrangement. The phasmid of the male can be located with the help of the female, which also has this structure on its tail. (Fig. 1, D.)

Measurements

<i>Measurements</i>					
1.9	14	18	20	17	78
1.5	2.9	3.4	46'	3.8	2.2
			48		0.9 mm. (0.86-0.94 mm.)
2.2	16	18	M	79	
2.1	3.2	3.5	41	3.2	0.75 mm. (0.68-0.81 mm.)

Diagnosis.—Diplogasteroides without lateral wings or longitudinal striae but with transverse striae composed of dots which are slightly larger on the lateral fields; without special labial structures, amphids near the end of the first fourth of the pharynx; anterior esophageal bulb well developed; female apparatus amphidelphic; vulva in front of middle; arrangement of male copulatory papillae as shown in Figure 1, C.

DIPLOGASTER AMERICANUS, N. SP.

Diplogaster americanus (figs. 2 and 3) is a large form with a long slender tail. The body tapers only slightly toward the head end, which is broadly convex. The cuticle is annulated at the head end as shown in Figure 2, A; but this annulation soon disappears and 24 longitudinal wings or ridges arise, which can be seen all along the body surface to the beginning of the filiform portion of the tail. Some of these wings fade out as the body begins to taper in the anal region. All the wings are equidistant and appear finely transversely striated, whereas the surface between them seems smooth. This is very strange, because the annulation in front of the wings is complete and continuous. With high magnification this anterior annulation resolves into transverse series of dots, but no such dots are seen on the transversely striated wings. Here, however, the annules are much finer. The dots on the head region are arranged in two series to each annule, one series in the interannular groove and one along the middle of each annule. A single circle of 6 papillae crowns the head, but in addition there are 16 small lips. In the specimens studied they appeared as small rounded elevations surrounding the oral opening and were easily seen in the side as well as in the frontal view. However, it is thought that these lips, when opened and unfolded, will present a different aspect. From the center of each a long, conical, setalike structure runs down the wall of the mouth cavity, giving the latter the appearance of being longitudinally striated. However, it is

thought that all these are labial papillae or setae, normally folded back into the oral cavity, but unfolded and protruded when the nema is feeding. Perhaps they are not sensory organs at all, but of mechanical significance during the intake of food. The pharynx is of irregular shape, the anterior part being wider than the posterior. There is a large slightly subdorsal tooth, curved forward, with strong muscles attached at its base; it is situated at the bottom of the anterior portion of the pharynx. This dorsal tooth, in side view, seems to be opposed by two submedial ventral ones of smaller size and not

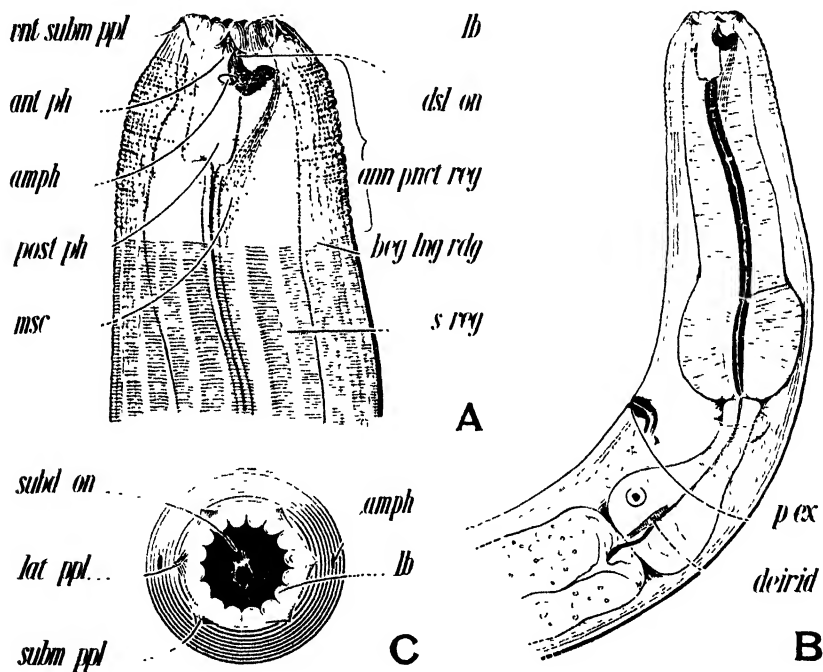


FIGURE 2 *Diplogaster americanus*, n. sp.

- A.—Head end in side view. *amph*, Amphid; *ann puct reg*, annulated and punctate region of cuticle; *ant ph*, anterior portion of pharynx; *beg lng ridg*, beginning of the cross-striated longitudinal ridges on the surface of the cuticle; *dsl on*, dorsal onchium; *lb*, labium (16); *msc*, protruding muscle of onchium; *post ph*, posterior portion of pharynx; *s reg*, smooth surface ribbon between the longitudinal ridges; *subm ppl*, ventrosubmedial papilla. \times about 1,433.
 B.—Anterior end in side view. *deirid*, Deirid located on a longitudinal ridge that is slightly enlarged at this place; *p ex*, porus excretorius. \times about 533.
 C.—Front view of head. *amph*, Amphid; *lat ppl*, lateral papilla; *lb*, labium; *subd on*, subdorsal onchium; *subm ppl*, submedial papilla. \times about 1,433.

curved; but in a front view nothing could be seen of the latter, and it is therefore concluded that these are only elements of the wall and not real teeth. The anterior portion of the esophagus is extremely strong and thick and followed by a large and muscular median bulb, distinctly set off and with well-developed strong internal walls. The second portion of the esophagus is shorter, well set off from the median bulb, of clear transparent appearance, and with a good-sized posterior bulb containing several nuclei. The intestine consists of large, granulated, polyhedral cells; there are probably only three in a cross section. The excretory pore is well defined and has strongly cutic-

ularized walls; it opens ventrad of the nerve ring. The female sexual apparatus is amphidelphic. The ovaries are reflexed. The male has a single reflexed testis. The male copulatory apparatus consists of two slender spicula, curved and slightly cephalated at the proximal ends. There is a single gubernaculum not quite half as

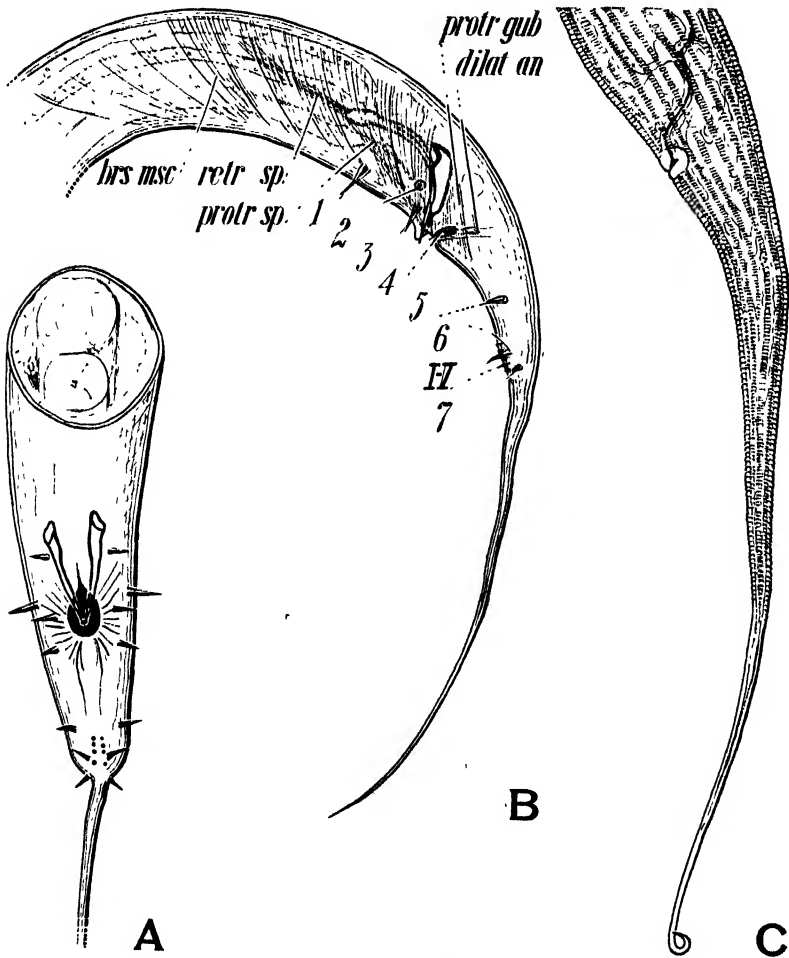


FIGURE 3.—*Diplogaster americanus*, n. sp.

A.—Ventral view of anal region of male, with copulatory apparatus (spicula, gubernaculum, and papillae). \times about 533
 B.—Tail end of male; *brs msc*, bursal muscles, *dilat an*, dilator ani; *protr gub*, protractor gubernaculi; *protr sp*, protractor spiculi; *retr sp*, retractor spiculi; 1-7, large copulatory papillae; 1-1', small copulatory papillae. \times about 533
 C.—Tail end of female. \times about 533

long as the spicula but much wider and stronger. (Fig. 3, A and B.) The arrangement of the muscles of this copulatory apparatus is best studied on Figure 3, B. The retractor spiculi has its usual position. One protractor spiculi runs from the proximal end of the spicula to the anterior labium of the anus. Another protractor muscle connects the proximal end of the spicula with the inner end of the gubernaculum.

The protractor gubernaculi connects the gubernaculum with the posterior labium of the anus. Numerous bursal muscles are also present, extending at least three to four times the length of the spiculum forward from the anal opening. The male copulatory papillae are very prominent; in fact they have more the shape of strong setae than of papillae. Some are much larger than the others. For their arrangement see Figure 3, A and B. As may be seen, all are ventrosubmedial or ventrolateral.

The present form is undoubtedly closely related to *Diplogaster striatus*, *D. viratus*, and *D. trichuris*. It belongs to the same group as these and may possibly later be brought into one and the same genus or subgenus, when the numerous species of the present genus *Diplogaster* are revised and reclassified.

Ecologically the members of the present genus *Diplogaster* include free-living, plant-parasitic, and animal-parasitic forms. "Bridges" exist between the free-living members and the others through saprophytic and saprozoic forms.

Measurements.

3.6	(12)	?	17	24	18	75	0.85 mm.
3.4	(3.9)	?	4.5	'46'	5.7	2.9	
2.2	(12)	?	19	47	'M	74	0.57 mm.
2.4	(5.1)	?	5.4		6.1	4	

Diagnosis.—*Diplogaster*, the pharynx of which is divided into a wide but short anterior portion at the base of which a large movable slightly subdorsal tooth found, and a less wide but not narrow cylindrical unarmed posterior portion about one and one-half times as long as the anterior. Lips small, 16 in number, with setalike processes, which can be folded into the anterior portion of the pharynx and appear then as longitudinal striae of the pharyngeal wall. Cuticle of the head end annulated, each annule with two transverse striae, one in the interannular groove, the other on the center of the annule; these striae resolve into dots; behind the head end 21 longitudinal, equidistant wings or ridges, which are finely transversely striated, whereas the space between them is smooth; walls of excretory pore heavily cuticularized. Male copulatory papillae as shown.

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SYNTHETIC NUTRIENT SOLUTIONS FOR CULTURING USTILAGO ZEAЕ¹

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INTRODUCTION

Ustilago zeaе (Beckm.) Ung., the organism causing smut of maize, when cultured artificially, usually is grown on a medium that has as a basis a decoction, extract, or infusion of a part of some plant or plant product. Various salts or sugars or both may be added. Potato decoction or carrot decoction or agar is generally used at present as the basis for media in which to culture the fungus. Sartoris² recommended a malt extract to which was added peptone, calcium nitrate, and potassium nitrate. Christensen and Stakman,³ in their work on physiologic specialization and mutation in *U. zeaе*, used a potato-dextrose medium.

These various infusions and decoctions are mixtures of soluble plant substances in solution. The specific substances contained in them are unknown for the most part, and of necessity they must be variable, depending upon the variety, conditions of growth, duration of storage, etc., of the material used. It is obvious that such culture media can not be standardized readily and that their exact duplication is uncertain. This is true whether the standardization and duplication are undertaken by different workers in the various laboratories or by the same worker in the same laboratory.

The observations and experiments that have indicated the existence of physiologic specialization in *Ustilago zeaе* have preceded the development of cultural media that could be accurately duplicated. It is known that these various physiologic forms differ greatly in appearance when cultured on different media. Most of the results on physiologic forms are based on morphologic characteristics of the fungus in culture. Carrot or potato decoction made from freshly grown material, for example, when compared with a decoction of the same name made from material that has been in storage for several months, may be so different in its physical or chemical properties as to be actually a different medium. A definite conception of physiologic forms of the corn-smut fungus thus grown would be difficult to establish. The results obtained might lead to confusion and the needless multiplication of supposedly different forms. Obviously there is need for some standard culture medium that can be accurately duplicated in any laboratory at any time.

Theoretically, the ideal medium for culturing *Ustilago zeaе* should satisfy the following requirements at least: (1) Its constituents

¹ Received for publication Oct. 1, 1929; issued October, 1930.

² SARTORIS, G. B. STUDIES IN THE LIFE HISTORY AND PHYSIOLOGY OF CERTAIN SMUTS. Amer. Jour. Bot. 11: 617-647, illus. 1924.

³ CHRISTENSEN, J. J., and STAKMAN, E. C. PHYSIOLOGIC SPECIALIZATION AND MUTATION IN USTILAGO ZEAЕ. Phytopathology 16: 979-990, illus. 1926.

should be pure chemicals that would permit accurate duplication of the medium; (2) it should be able to produce, in the same period of time and under the same conditions, an equal or a greater quantity of growth of *U. zeae* than is produced in the generally used potato or carrot decoction; (3) it should have as few constituents as possible consistent with this growth-producing standard; and (4) the constituent chemicals, when added in solution and in culture concentration, should produce little or no precipitate, and the resulting medium should have a hydrogen-ion concentration (preferably after sterilization) favorable to growth without the addition of either acid or alkali to adjust the reaction.

With the above-listed four requirements as a guide, a great many combinations were made. Many were ruled out because of the formation of precipitates that were not dissolved following sterilization of the culture solution. Other combinations were discarded because of unfavorable hydrogen-ion concentration either before or after the growth of the fungus in them. Finally 26 nutrient combinations were chosen for a more detailed study.

MATERIALS AND METHODS

The 26 nutrient solutions listed in Table 1 were tested in detail for growth-producing possibilities in comparison with the control medium, carrot decoction. Most of these solutions were free from precipitates after sterilization, but a few of them produced a negligible precipitate. These nutrient solutions are referred to as "synthetic" in contrast to the decoction mixtures now used generally.

TABLE 1.—Formulas of 26 synthetic solutions tested for culturing *Ustilago zeae*
[Quantities of chemicals expressed as grams in a liter of solution]

Nutrient solution No.	Quantity of indicated chemical																				Dextrose
	K ₂ HPO ₄	KH ₂ PO ₄	KCl	NaNO ₃	MgSO ₄ ·7H ₂ O	KNO ₃	NaCl	Ca (NO ₃) ₂ ·4H ₂ O	K ₂ SO ₄	NH ₄ NO ₃	CaCl ₂	Na ₂ SO ₄	Na ₂ HPO ₄ ·12H ₂ O	CaSO ₄ ·2H ₂ O	CaH ₂ (PO ₄)·2H ₂ O	Ferric phosphate soluble scales	MnSO ₄ ·2H ₂ O	Mg ₂ (PO ₄) ₃ ·4H ₂ O	FeCl ₃		
1	0.25			0.15	0.25	0.2															10
2	.3			.2	.25																10
3		0.25				15.0	1	0.15		0.1											10
4	.3				.2	.1		.1													10
5		15	.2		.2	.15															10
6	.2		.2		.2																10
7																					10
8																					10
9																					10
10																					10
11		.2			.2	.2	.2														10
12		.2			.2	.2	.2														10
13		.2			.2	.2	.2														10
14		.2			.2	.2	.2														10
15		.2			.2	.2	.2														10
16		.2			.2	.2	.2														10
17		.2			.2	.2	.2														10
18					.2	.25															10
19					.2	.25															10
20								.2	.2										.2	0.1	10
21					.2	.2														.1	10
22								.2	.2										.2		10
23					.2	.2													.1		10
24								.2	.2										.2	.1	10
25					.2	.2													.1	.1	10
26				.2					.3		.1							.1			10

The chemicals employed in the culture solutions given in Table 1 were measured in cubic centimeters from stock solutions. The stock solutions of the first 13 chemicals, K_2HPO_4 to $Na_2HPO_4 \cdot 12H_2O$, inclusive, were of such concentration that a unit of 10 c. c. was equivalent to 1 gm. of the salt. The stock solutions of the next 6 chemicals, $CaSO_4 \cdot 2H_2O$ to $FeCl_3$, inclusive, were of such concentration that a unit of 100 c. c. was equivalent to 1 gm. of the salt. The stock solution of dextrose contained 200 gm. per liter, i. e., a unit of 5 c. c. was equivalent to 1 gm. of sugar.

The same stock solutions were used throughout the experiments, and the same pipettes were used in measuring the required quantities of the respective stock solutions. In making up the various culture solutions about 800 c. c. of distilled water was added to the flask prior to the addition of any of the chemical constituents. This was done to minimize the formation of precipitates. With the exception of solutions Nos. 21 and 25, all those containing both magnesium and iron produced a slight flocculent colloidal precipitate. This precipitate could be resuspended readily on shaking. All other solutions contained a negligible precipitate or none at all after sterilization.

The control solution was a carrot decoction. This was chosen as a standard of measure because of its general use in culturing *Ustilago zae*. It was made from chopped raw carrots in distilled water. This mixture was autoclaved for 30 minutes at a pressure of 15 pounds, after which the mass was filtered and the filtrate was diluted with distilled water until it was a decoction of 1,000 gm. of carrot roots per liter of solution.

TABLE 2. Hydrogen-ion concentrations of the 26 nutrient solutions and of the control, both before and after sterilization

Nutrient solution No	pH in relation to sterilization		Nutrient solution No	pH in relation to sterilization	
	Before	After		Before	After
1	5.9	5.5	14	5.0	4.4
2	5.7	5.4	15	5.3	4.9
3	5.2	4.3	16	5.1	5.0
4	7.2	5.7	17	5.1	4.1
5	6.8	4.0	18	7.0	5.8
6	5.2	5.0	19	6.9	6.2
7	7.4	5.6	20	5.8	4.5
Control	5.0	5.0	21	3.1	3.0
8	7.5	5.5	22	7.0	6.1
9	4.3	4.0	23	4.9	4.1
10	5.0	4.4	24	4.9	4.4
11	4.9	4.8	25	3.1	3.0
12	5.3	4.9	26	7.5	6.0
13	5.0	4.4			

The hydrogen-ion concentrations of the 26 nutrient solutions and of the control (carrot) decoction, before and after sterilization, are given in Table 2. The determinations were made colorimetrically.

After the various nutrient solutions were prepared, 50 c. c. of each was pipetted into each of the desired number of 125 c. c. Erlenmeyer flasks and sterilized in the autoclave for 15 minutes at a pressure of 15 pounds. After sterilization, three to five flasks of each of the nutrient solutions and of the control were inoculated with the smut from a single collection.

Smuts from nine different collections were used. Data on these are given in Table 3. These smuts were grown separately in carrot decoction for six days, during which time they were constantly agitated in a mechanical shaker to prevent growth-lump formation. They were then used as the inoculum, one drop being used to inoculate each of the flasks containing 50 c. c. of the various nutrient solutions. After inoculation the flasks were placed in an incubator maintained at a temperature of $25^{\circ}\text{C.} \pm 0.5^{\circ}$. Twice each day the flasks were shaken by hand to keep the smut colonies broken up and thus prevent formation of growth lumps.

TABLE 3.—History and source of the nine cultures of *Ustilago zae* used in the experiments on media

Culture No.	History and source
1ss-----	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1921 from Madison, Wis.
2ss-----	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1922 from Arlington Experiment Farm, Rosslyn, Va.
4ss-2----	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1923 from Arlington Experiment Farm.
35ss----	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1925 from Arlington Experiment Farm.
3-2-----	Isolated from smut-gall tissue collected at Clemson College, S. C., 1927
34-2-----	Isolated from smut-gall tissue collected at Mesilla Park, N. Mex., 1927
50-1-----	Isolated from smut-gall tissue collected at the Davis County Experiment Farm, Farmington, Utah, 1927.
60-2-----	Isolated from smut-gall tissue collected at Manhattan, Kans., 1927
74-2-----	Isolated from smut-gall tissue collected at University Farm, St. Paul, Minn., 1927.

Each smut was grown in the various nutrient solutions for the periods of time indicated in Table 4. At the end of the period the flasks were removed from the incubator and the quantity of growth that had developed was determined.

Considerable difficulty was experienced in finding a method that would be sufficiently accurate and yet rapid enough to allow making the determinations within a reasonable time. The method of determination by filtration and subsequent weighing of the smut residue and filter paper was found to be impossibly slow. The method of evaporating the liquid from the cultures and then weighing the smut and remaining solids was considered too inaccurate because of the salt and sugar residue that would be weighed with the smut. This error would be greatest with those cultures that had grown least and thus would tend to equalize the results from all solutions.

A combined method, consisting of centrifugation followed by filtration of the supernatant liquid and subsequent weighing of the total residue, was tried. This method was reasonably accurate, but it was slow, and for routine work there were too many operations with the danger of loss of material during transfer. It was found also that this combined method did not produce results that were any more accurate or more uniform than did the method finally selected.

This method consisted of centrifugation at 1,800 revolutions per minute for three minutes, followed by the direct reading of the quantitative results in terms of cubic centimeters of smut thrown down. This centrifuge method was rapid and as accurate as any method tried. Centrifuge tubes were used that were accurately calibrated to 0.10 c. c. and that could be estimated to 0.05 c. c. All the

results are reported as "cubic centimeters of growth obtained by centrifugation."

TABLE 4.--Average growth of cultures of *Ustilago zeae* in the control (carrot decoction) and in the 26 synthetic nutrient solutions listed in Table 1

[Each figure, which is the average of three to five cultures, indicates the cubic centimeters of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m. The incubation periods were as follows. No. 3-2, 2 weeks, No. 1ss, 10 days, all others, 3 weeks]

Nutrient solution No.	Average fungus growth produced by cultures of the indicated number									
	2ss	4ss-2	1ss	35ss	3 2	34-2	50-1	60-2	74-2	
1.	0.5	1.7	0.3	0.4	0.2	0.2	0.2	0.3	0.1	
2.	.5	1.4	.4	.5	.2	.4	.1	.2	.1	
3.	.5	1.7	.1	.4	.3	.2	.1	.2	.1	
4.	.2	1.1	.2	.6	.1	.2	.1	.2	.05	
5.	.6	1.1	.1	.2	.2	.2	.1	.2	.1	
6.	.1	.7	.1	.2	.1	.1	.1	.1	.1	
7.	.7	1.8	.4	.7	.2	.5	.2	.4	.2	
8.	.6	1.0	.1	.2	.1	.1	.05	.3	.05	
9.	.5	1.3	.1	.3	.1	.2	.2	.2	.1	
10.	.5	1.2	.3	.4	.1	.2	.1	.3	.1	
11.	.6	1.1	.6	.5	.3	.3	.05	.3	.1	
12.	.4	1.4	.7	.5	.3	.3	.1	.3	.1	
13.	.1	1.4	.1	.3	.2	.2	.1	.3	.1	
14.	.3	1.1	.1	.4	.2	.2	.1	.2	.05	
15.	.3	1.4	.5	.3	.3	.3	.05	.3	.1	
16.	.3	1.3	.5	.5	.1	.3	.05	.3	.1	
17.	.4	1.6	.5	.5	.3	.2	.1	.3	.1	
18.	.4	1.5	.5	.5	.1	.2	.1	.1	.1	
19.	.1	1.6	.7	.6	.2	.4	.1	.3	.1	
20.	.4	1.0	.4	.5	.1	.1	.1	.3	.1	
21.	.5	1.1	.8	.2	.1	.2	.05	.2	.1	
22.	.2	.6	.1	.3	.1	.2	.1	.4	.05	
23.	.5	1.2	.2	.4	.2	.2	.1	.5	.05	
24.	.4	1.1	.2	.4	.2	.1	.05	.1	.05	
25.	.6	1.0	.8	.3	.2	.3	.05	.2	.1	
26.	.5	.5	.4	.2	.2	.3	.1	.3	.1	
Control	.4	1.3	.5	.1		.2	.1	.4	.1	

RESULTS OBTAINED WITH VARIOUS SYNTHETIC SOLUTIONS

The data obtained by growing the various smut cultures in the 26 nutrient solutions tested and in the control (carrot decoction) are given in Table 4. In this table is shown the average growth of each smut in each nutrient solution. There are 242 averages to be compared, 1 being missing from the total of 243. Of these, and for each respective smut, 226 of the average quantities of growth were less than or only equal to those produced in solution No. 7. In only 16 of the 242 averages were the respective average quantities of growth greater in some other nutrient solution than in No. 7. In all 16 cases in which the average growth was less in solution No. 7 than in some other nutrient solution the smut culture was either No. 1ss or No. 3-2. In but 1 case (smut No. 1ss) did the average growth in the control solution (carrot decoction) exceed that in solution No. 7.

An analysis of the data in Table 4 indicates, therefore, that synthetic nutrient solution No. 7 is a superior medium for culturing *Ustilago zeae*. A consideration of Figure 1 confirms this indication. In Figure 1 the average growth of all nine smut cultures in each of the respective nutrient solutions is graphically represented. The data on total growth rather than on the average growth of each smut culture in the respective solution bear out the same conclusion. Such a comparison could have been graphed had it not been for the inability to obtain the results of growth of smut No. 3-2 in the control solution (Table 4). This culture was too viscous to centrifuge quantitatively.

In order to determine further the value of nutrient solution No. 7, the latter was used in a second comparative test with carrot decoction. The test was made under conditions similar to those of the

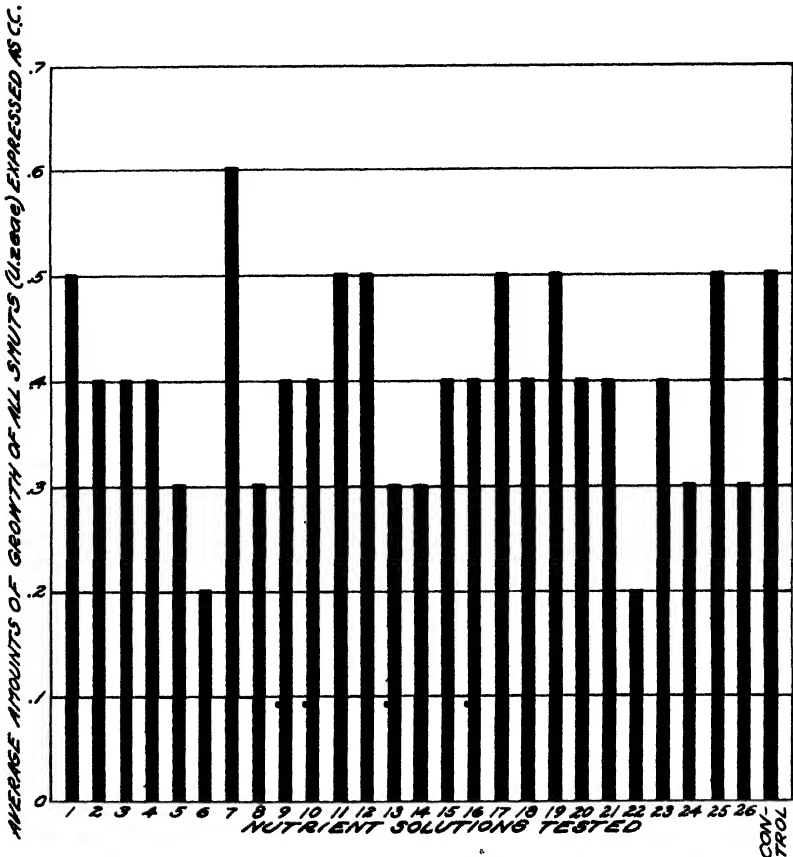


FIGURE 1.—Average growth of 9 collections of corn smut (*Ustilago zeae*) cultured in the control solution (carrot decoction) and in the 26 synthetic nutrient solutions tested. These are averages of totals and necessarily differ somewhat from similar averages calculated from the data of Table 4

first test, except that seven instead of nine cultures of *Ustilago zeae* were used. The results are presented in Table 5.

TABLE 5.—Average growth of cultures of *Ustilago zeae* in the control (carrot decoction) and in synthetic nutrient solution No. 7

[Each figure, which is the average of five cultures, indicates the cubic centimeters of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m. The incubation period was three weeks]

Nutrient solution	Average fungus growth produced by cultures of the indicated number *						
	1ss	2ss	4ss-2	35ss	34-2	50-1	74-2
No. 7.....	0.5	0.6	1.7	0.8	0.3	0.2	0.2
Control.....	.5	.5	1.1	.4	.2	.2	.1

* Smut cultures Nos. 3-2 and 60-2 are not included because they did not survive and were not available when the work was repeated.

Table 5 shows that the fungus growth of two of the cultures was the same in solution No. 7 and in carrot decoction and that the growth of five of the cultures was greater in nutrient solution No. 7 than in carrot decoction. This confirms the data of Table 4 in showing the value of nutrient solution No. 7.

OPTIMUM DEXTROSE CONTENT

It will be noted that all the nutrient solutions tested, except the control, contained uniformly 10 gm. of dextrose per liter of solution. As this quantity of dextrose was chosen arbitrarily, the desirability of determining the optimum quantity of dextrose is apparent.

As solution No. 7 had proved to be the one best suited for culturing the organism, it was used as the culture medium in tests for this purpose. The dextrose content was varied from 2.5 to 30 gm. per liter, forming 10 nutrient solutions as indicated in Table 6. Flasks containing 50 c. c. of each solution were used, as previously described. Each 50 c. c. lot was inoculated with one drop of a carrot-decoction culture of smut No. 4ss-2, generally the most rapidly growing culture in the group, but for some unknown reason not always constant in its growth responses under supposedly identical conditions. The contained cultures were incubated at a temperature of $25^{\circ} \pm 0.5^{\circ} \text{C.}$, and twice each day the flasks were shaken to prevent colony formation. At the end of 3, 6, and 9 weeks, respectively, the quantity of growth in the three sets of five flasks of each solution was determined by centrifugation as previously described. The results are given in Table 6 as averages of the five flasks of each series.

TABLE 6. -Results of tests to determine the optimum amount of dextrose to use in synthetic nutrient solution No. 7 for culturing *Ustilago zae*

[Each figure indicates the cubic centimeter of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m.]

Culture solution No.	Dextrose (grams per liter)	Average growth of smut culture No. 4ss-2, after indicated period			Culture solution No.	Dextrose (grams per liter)	Average growth of smut culture No. 4ss-2, after indicated period		
		3 weeks	6 weeks	9 weeks			3 weeks	6 weeks	9 weeks
7-a-----	2.5	1.1	1.0	1.0	7-e-----	15.0	0.8	0.7	1.0
7-b-----	5.0	1.3	1.7	1.9	7-f-----	17.5	.7	1.0	1.0
7-c-----	7.5	1.1	1.9	2.0	7-g-----	20.0	.6	.8	.8
7-d-----	10.0	1.0	1.7	1.8	7-h-----	25.0	.5	.6	.5
	12.5	.9	1.1	1.5	7-i-----	30.0	.5	.5	.5

From the data of Table 6 it is evident that growth is definitely limited by the addition of less than 5 gm. or more than 10 gm. of dextrose per liter of culture solution. The optimum quantity appears to vary from 5 to 10 gm. of dextrose per liter, depending upon the length of time the culture is incubated.

COMPARISON OF DEXTROSE WITH MALTOSE

Using maltose, Sartoris ⁴ found that the optimum concentration for culturing *Ustilago zae* and certain other species of the same genus was M/7, as shown in his Table 5. He used this concentration (M/7)

⁴ SARTORIS, G. B. Op. cit.

in a study of the relative influence of dextrose, lactose, levulose, and maltose on the growth of certain smut species, including *U. zeae*. A solution of M/7 concentration would correspond to approximately 51.5 gm. per liter for a disaccharide ($C_{12}H_{22}O_{11} \cdot H_2O$) and to approximately 28.3 gm. per liter for a monosaccharide ($C_6H_{12}O_6 \cdot H_2O$). This concentration (28.3 gm. per liter) of dextrose used by Sartoris is from 2.8 to 5.7 times as concentrated as the optimum concentration (5 to 10 gm. per liter) found by the writer in growing corn-smut culture No. 4ss-2. The results obtained by the writer can not be compared directly with those obtained with *U. zeae* by Sartoris (his Tables 5 and 6) because his data are not reported in quantitative units of measure. The quantities of growth obtained in his experiments were determined, apparently, by observation and reported according to arbitrarily chosen scales, the minimum of which was indicated by the figure 1 and the maxima of which were indicated by the figures 4 or 5.

Sartoris⁵ found that maltose was the best sugar for the development of secondary spores of *Ustilago zeae*. As dextrose had been used as the source of carbohydrate throughout these experiments, it was decided to compare the growth of smuts in nutrient solution No. 7 containing dextrose with the growth of smuts in the same solution in which maltose had been substituted for the dextrose. Fifty cubic centimeters of each solution was used per 125 c. c. flask, and triplicate cultures for all the smuts used previously were made in each of the two solutions. The cultures were incubated and shaken as indicated in previous experiments. After three weeks the quantities of growth were determined by centrifugation and are expressed as cubic centimeters of smut thrown down. These data are given in Table 7.

TABLE 7.—Average growth of seven smut cultures (*Ustilago zeae*), each incubated three weeks in five flasks of nutrient solution No. 7, containing either dextrose or maltose at the rate of 10 grams per liter

[Each figure indicates the cubic centimeters of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m.]

Nutrient solution No 7 containing --	Average growth of culture No						
	1ss	2ss	4ss-2	35ss	34-2	50-1	74-2
Dextrose.....	0.6	0.7	1.6	0.7	0.5	0.3	0.2
Maltose.....	1.1	8	2.0	1.0	.6	.2	.2

The data of Table 7 indicate that with five of the smut cultures maltose supported more growth than did dextrose. In one case the quantity of growth was the same, and in one case dextrose supported more growth. These data indicate also the variable carbohydrate metabolism of the various smut cultures, the variation being most common in those cultures each of which was derived from a single sporidium (Nos. 1ss, 2ss, 4ss-2, and 35ss).

⁵ SARTORIS, G. B. Op. cit.

CONCLUSIONS AND SUMMARY

The various nutrient media (decoctions, extracts, or infusions of plant parts or plant products) generally used for culturing *Ustilago zeae* are unsatisfactory. This is true especially in corroborative work or repeated experiments leading to the possible segregation, classification, and identification of the various physiologic forms of *U. zeae*. It is no less true in experiments dealing with the fundamental nature of resistance of corn to infection by *U. zeae*. There is need, therefore, for a synthetic nutrient medium composed only of definite, purified, chemical compounds, thus permitting accurate duplication at any time and in any laboratory.

The data reported show that synthetic nutrient solution No. 7, described herein, meets this need and satisfies the requirements for such a medium. Experiments with nine separate smut cultures (*Ustilago zeae*) have demonstrated its superiority over carrot decoction and the other solutions tested. Nutrient solution No. 7 is composed of but four inorganic compounds and dextrose in solution, to which agar may be added to produce a solid medium. When these constituents are added in solution there is practically no precipitate formed and the resulting hydrogen-ion concentration is favorable to growth. This situation makes it possible to keep the total concentration of the solution at a constant level and permits accurate duplication, as neither acid nor alkali need be added to adjust the reaction of the solution. The reaction of this nutrient solution before sterilization is pH 7.4, and after sterilization it is pH 5.6.

Synthetic nutrient solution No. 7 has the following composition, expressed as grams per liter of solution:

K ₂ SO ₄	0.3 gm.
NH ₄ NO ₃1 gm.
Ca. Cl ₂1 gm.
Mg ₃ (PO ₄) ₂ .4H ₂ O1 gm.
Dextrose	10.0 gm.
Distilled water to make	1.0 liter

When used as a solid medium, 1.5 per cent of agar was added. For certain physiologic forms of smut (*Ustilago zeae*), it may be desirable to substitute maltose for dextrose. Maltose, however, is from 10 to 15 times as expensive as dextrose.

PREDACIOUS NEMAS OF THE GENUS NYGOLAIMUS AND A NEW GENUS, SECTONEMA¹

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INTRODUCTION

The major nemie pests of cultivated crops in this country have no important natural enemies, at least so far as is known at the present time. This seems to be especially true of the sugar-beet nematode, *Heterodera schachtii* Schmidt, which is one of the most destructive parasites of the sugar beet in the Western States. Many billions of these nemas frequently are found in an acre of ground, and it would seem that such enormous numbers would attract some natural enemies. Predacious nemas of the genus *Mononchus* occasionally prey upon them, but so far as is known, not to a degree that is of economic importance (8).³

Among the other predacious species commonly found in these infested fields are those belonging to the genus *Nygolaimus* Cobb (1, 2) and *Sectonema*, nov. gen. These two groups were the subject of the researches here recorded.

NYGOLAIMUS

HABITS

FOOD HABITS

For this study fields were selected that were inhabited by several species of nygolaims and heavily infested with *Heterodera schachtii*. Soil samples were collected from about the beet roots and the nemas secured by sifting and gravity methods. Many nygolaims were found living in close proximity to the females of *H. schachtii* that were clinging to the beet roots. These females apparently offered ideal victims for the nygolaims, for, being attached to the beet roots, they could not escape. Hundreds of specimens were collected and the intestines carefully examined for traces of the body contents of *H. schachtii* or other nemas, but in not a single instance were such remains found.

In many of the specimens examined it was possible to identify the setae and cuticle of oligochaete worms (Enchytraeidae), and practically all individuals contained material that appeared to be the body contents of these worms. This was especially true of small species like *Nygolaimus vulgaris*, which are too small to swallow anything except the liquid or granular contents of the worm's body.

When fed in the laboratory both nygolaims and sectonemas invariably refused to devour other nemas but frequently attacked and fed

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² The writer is deeply indebted to Dr. N. A. Cobb and Dr. G. Steiner, of the Office of Nematology, for helpful criticisms and suggestions during the preparation of this paper. Thanks are also due Dr. J. G. de Man, of Ierseke, Netherlands, for verifying the identification of *Nygolaimus brachyurus* and *N. hartingii*.

³ Reference is made by number (italic) to Literature Cited, p. 465.

upon the oligochaetes. In this respect *Sectonema ventralis* was found to be more voracious than any of the nygolaims.

METHOD OF ATTACK

The nygolaims apparently located the oligochaete by touch only, or at least by very close proximity. Often the head of the nygolaim may pass very near the worm without the nygolaim's appearing to observe its presence. When the nygolaim locates the oligochaete it presses its lips tightly against the body, the lips probably being held there by the suction of the esophagus. The pharynx is then thrust forward, as shown in Figure 8, A, B, C, and the tooth driven into the body of the victim. The tooth is then withdrawn and the body contents of the prey sucked out. The writer has not observed a feeding nygolaim devour the skin of a victim, but that this frequently is done, at least by the larger species, is shown by the many specimens that contain the setae and cuticle of oligochaetes in the intestine.

From the knowledge at present available it appears that the nygolaims are not of economic importance in the control of plant-infesting nemas.

HABITAT

The nygolaims inhabit both soil and fresh water, but at present those in the soil appear much the more numerous. Like the predacious mononchs they prefer the lighter sandy soils, probably for the reason that sandy soils give more freedom of movement for pursuit and capture of their prey. Occasionally, however, colonies of the smaller species are observed in clay loams. Some species are found only where there is an abundance of moisture, while others prefer the arid desert soils in which activity is limited to only a few weeks in the spring and fall when the soil is moistened by rains. Apparently there is only one generation each year, most of the eggs being deposited during April, May, and June, although rarely gravid females are found during other months.

The distribution of the various species in a locality is usually quite definite. Some are found in the cultivated valley soils, while others appear only in collections from the mountains. The largest populations usually occur in the cultivated fields.

In no instance has the same species been found inhabiting both cultivated and virgin soil. The native species disappear when the land is cleared and irrigated, and in their place are found species whose origin is problematical. Near Salem, Utah, *Nyggolaimus tenuis* and *N. vulgaris* are numerous in cultivated fields, while in virgin soil only a few rods distant *N. brachyurus* and *N. bisexualis* are the species found.

Nyggolaimus vulgaris, *Sectonema ventralis*, and *Oligochaeta* were present in the two fields located near Lewiston and Salem, Utah, where the studies were made on the mononch populations (8). *N. tenuis* was also present in the Salem field, while *N. brachyurus* and *N. amphigonius* were studied in virgin soil near this field. The vertical range of habitat for these species is given in Table 1. The numbers of individuals are the totals counted for each species and are not comparable as to the relative numbers actually present in the soil, because only two 10000-acre samples were examined for *N. brachyurus* and *N. amphigonius*, while 34 samples were examined for *N. vulgaris* and 30 for *N. tenuis*.

TABLE 1.—Vertical range in the soil of *Nyggolaimus* (four species) *Sectonema ventralis*, and *Oligochaeta*

Depth	<i>Nyggolaimus vulgaris</i>	<i>N. tenuis</i>	<i>N. amphigonius</i>	<i>N. brachyurus</i>	<i>Sectonema ventralis</i>	<i>Oligochaeta</i>
Inches	Number	Number	Number	Number	Number	Number
3-2	721		7		2	197
1-4	922		4	11	24	420
5-6	516		1	10	20	439
7-8	235			6	19	407
9-10	142	1		4	12	196
11-12	80	22		2	6	134
13-14	48	14			13	64
15-16	2	12			2	64
17-18	5	5			7	64
19-20	4	7			1	37
21-22		4			1	34
23-24		2				20
Total	2,675	67	12	33	107	2,076

The population per acre for the following species was computed from representative soil samples; *Nyggolaimus vulgaris*, 78,888,000; *N. tenuis*, 2,250,000; *N. amphigonius*, 6,000,000; *N. brachyurus*, 16,500,000; *Sectonema ventralis*, 6,727,000; *Oligochaeta*, 107,700,000.

It is evident that all these species, except *Nyggolaimus tenuis*, generally are found in the first foot of soil, as is common with most nemas. Unfortunately, no data are available on other species collected by the writer, but these are from mountain or desert soil from which the samples usually were taken to a depth of less than 1 foot; so it may be concluded that, of the species studied, *N. tenuis* is the only one that favors a deep habitat. In no instance has there been observed any tendency for a species to vary its depth of habitat in response to changes in moisture or temperature. The above data are composite results of many samples taken at all months of the year, and include only a small portion of the individuals of these species that actually have been observed in the extensive soil-examination work carried on by the writer.

GENERAL STRUCTURE

In general appearance certain *nyggolaimus* so closely resemble nemas of the genus *Dorylaimus* that various nematologists have placed them in that genus without noting their true identity.

While the writer was examining a collection of what was supposed to be *Dorylaimus hartingii* his attention was attracted by the three cardiac glands and the dorsal organs crowding the anterior end of the intestine. These were so unusual in *Dorylaimus* that a more careful examination of the specimens was made, and it was found that the tooth was located on the left submedian wall of the pharynx. This immediately placed the species in *Nyggolaimus*, and a careful search through the writer's collection of about 90 species of *dorylaimus* revealed 10 more species of *Nyggolaimus* that previously had been overlooked.

During this investigation these two closely related genera were found to differ in the following respects:

Nyggolaimus

Tooth set on left submedian wall of pharynx.
 No definite guiding ring for tooth.
 Food does not pass through tooth.
 Pharynx deep and definitely set off.
 Three conspicuous glands at base of esophagus.
 Lips of vulva not cuticularized.
 Gubernaculum posterior to the spicula present.

Dorylaimus

Tooth submedian, but not so distinctly pharyngeal.
 Definite guiding ring for tooth present.
 Food passes through tooth.
 Pharynx not deep or definite.
 Glands not conspicuous at base of esophagus.
 Lips of vulva cuticularized.
 Gubernaculum posterior to the spicula not present.

BODY.—*Nyggolaimus* are comparatively large nemas, the length varying from slightly over 1 mm. in *Nyggolaimus dubius* to 4-5 mm. in *N. denticulatus* and *N. ferax*. The body is slender, the width rarely exceeding 2.5 per cent of the length and often being less. The neck is slightly convex conoid to the head. Posteriorly the body begins to taper some distance in front of the anus, beginning to decrease suddenly near the anus and ending in a hemispherical to elongate-conoid tail without a spinneret.

PORES.—The series of lateral pores described by Cobb for *Nyggolaimus denticulatus* doubtless is present in all species. In some of them, especially the smaller ones, they are extremely difficult to see, or even invisible. The anterior pore of the lateral series sometimes is located in the cuticle near the center of the escutcheon-shaped amphid. The first three or four pores lie almost in line, then the series begins to diverge into two distinct lines that continue nearly to the terminus. On *N. brachyurus* and others there are dorsal and ventral series reaching from the head nearly to the middle of the esophagus.

The phasmids apparently are not definite but are included in the lateral series of pores. However, there usually is one pore near the middle of the tail that is more prominent than the others, and this may be the phasmid proper.

HEAD.—The six confluent lips are amalgamated into a hexagonal mass bearing two circlets of innervated papillae that do not interfere with the contour of the lips. The inner circlet consists of 6 papillae. The outer circlet has only 6 in small species, but in the larger forms like *Nyggolaimus brachyurus* and *N. vorax* there are 10. From a front view the lips appear to lack lip flaps, and the entrance to the pharynx is closed by drawing the lips together. The broad amphids are escutcheon-shaped with obscure slitlike openings. Frequently the body twists until the amphids, seen dorsoventrally, give the head the appearance of being set off by a deep constriction, whereas in reality it may not be set off in any manner.

PHARYNX.—The pharynx opens through a narrow vestibule made up of six folds that allow for great expansion. (Fig. 7, B.) From the usual lateral view these plications frequently appear to form a guiding ring for the spear. The pharynx proper is of double structure, the anterior portion being somewhat fusiform and the posterior more or less tubular. The anterior portion is made up of three sections arranged tandem, the first extending from the vestibule and widening out to the full width of the pharynx. The second is hexagonal in structure and forms the main body of the pharynx. (Fig. 7, C.) The third portion is pentagonal, due to the fusing of the two left submedian plates into a solid mass to support the tooth. (Fig. 7, D.) This third section narrows to join the long, slightly triquetrous, tubular posterior

portion of the pharynx. These sections form the eversible portion of the pharynx and allow the tooth to be thrust forward out of the mouth as shown in Figure 8, A-C.

TOOTH.—The tooth of *Nyggolaimus denticulatus* and *N. menzelii* tends to be quite arcuate, but in the remainder of the species it is almost straight and projects directly forward from its location on the wall of the pharynx. Usually it is hollow almost the entire length, but in *N. vulgaris* and *N. bisexualis* the distal portion is an exceedingly slender, solid point.

ESOPHAGUS.—This organ is quite typically dorylaimoid. Usually the posterior three-fifths is enlarged by a gradual expansion, and frequently the enlarged portion is irregular in width. (Fig. 9.) The musculature is strongly developed and internal glands are present.

CARDIAC GLANDS.—These are one of the distinctive features of nyggolaims, and it is to be wondered at that they have not been noted by previous investigators. They closely resemble the glands of *Trilobus* in their size and position, one being ventral, the other two subdorsal. Their secretions enter the digestive tract through pores located at the junction of the esophagus and the intestine. (Fig. 3, C.)

A pair of conspicuous organs are present in *Nyggolaimus teres*, *N. dubius*, *N. acuticaudatus*, and *N. hartingii*. These are located subdorsally and crowd the anterior end of the intestine. (Figs. 12, D; 13, D; and 15, D.) Their function is problematical, but from the fact that ampullae extend forward from them and enter into large cells located in the lateral chords, it appears evident that they produce secretions. Their presence was noted by De Man in his original description of *N. hartingii*. The size of these organs varies considerably in individuals from the same collection, in some being less than the body width in length, in others four or five times as long as the body width.

DIGESTIVE TRACT.—The intestine is always distinctly set off from the esophagus and is made up of cells that require 6 to 10 for a circumference. The cells usually are well filled with rather uniform fine granules that often are light brown in color, frequently giving the body a rich brown appearance. The walls of the intestine are thin and allow observation of the contents of the lumen. The pre-rectum is short, usually being one to three times as long as the rectum. The rectum is from one to one and one-half times as long as the anal body diameter and ends in a conspicuous depressed anus with distinct lips.

REPRODUCTIVE SYSTEMS.—Males are known of only 7 of the 16 species of *Nyggolaimus*. That the females of at least some of the other species are syngonic may safely be predicted, since large collections made at various times of the year have invariably failed to contain males.

Two ovaries are present in all the known species. The gonads are of approximately the same length and together rarely occupy more than one-fourth of the body length. The ovaries are always reflexed.

There are two testes, of which the left is the longer and extends forward about half the length of the body and does not have the distal end reflexed. The right is the shorter of the two and is reflexed a distance equal to three or four times the width of the body. The testes contain several hundred spermatozoa of fairly large size. The

spicula are thick, strong, and slightly arcuate. They rest on short, thick gubernacula that sometimes are difficult to see because of their peculiar refractive index. Bifurcate lateral guiding pieces usually are present and are joined to the gubernacula by bands of muscles. The musculature of the male tail is not so heavily developed as in some genera. The series of male supplementary organs is variable, only a single pair of anal papillae being present in *Nygotaimus brachyurus*, while in *N. teres* and others there are as many as eight preanal supplements in addition to the pair of papillae just in front of the anus.

TAXONOMY

There follows a key to the species of *Nygotaimus*, together with descriptions of the 6 forms previously known and 10 hitherto undescribed species. These fall into two distinct groups, for which the subgeneric names *Nygotaimus* and *Nygotaimium* are here proposed. The various species are shown in Figures 1 to 17. All figures of heads are shown at a magnification of 1,000, while the tails and other parts of the body are shown at 500 unless otherwise stated.

KEY TO THE GENUS NYGOTAIMUS

	Subgenera and species	Figure No.
Pharynx without denticles:		
Type <i>N. pachydermatus</i> Cobb (1), 1913----	NYGOTAIMUS	
Terminus blunt and rounded.		
Lip region set off by a constriction.		
Tooth hollow less than half its length.		
Cuticle of tail with distinct outer layer-----	<i>vulgaris</i> , n. sp.	1
Cuticle of tail without distinct outer layer-----	<i>bisexualis</i> , n. sp.	2
Tooth hollow almost to apex.		
Length rarely over 2 mm.		
Lip region only one-third as high as wide-----	<i>pachydermatus</i> Cobb (1), 1913	5
Lip region one-half or more as high as wide		
Pharynx three times as deep as lip width-----	<i>tenuis</i> , n. sp.	3
Pharynx twice as deep as lip width-----	<i>amphigoniscus</i> , n. sp.	4
Length well over 2 mm.		
Tail hemispherical-----	<i>obtusius</i> , n. sp.	6
Tail not hemispherical, more conoid-----	<i>brachyurus</i> De Man (4), 1884	7
Lip region continuous with head contour.		
Length under 2 mm-----	<i>teres</i> , n. sp.	12
Length over 2 mm.		
Pharynx about four times as deep as lip width-----	<i>aquaticus</i> , n. sp.	8, 9
Pharynx less than three times as deep as lip width.		
Tail shorter than anal body diameter-----	<i>ferox</i> , n. sp.	10
Tail as long as anal body diameter-----	<i>shadini</i> Filipjev (3), 1928	11
Terminus acute or subacute.		
Tooth half as long as lip width-----	<i>acuticaudatus</i> , n. sp.	13
Tooth about as long as lip width.		
Tail twice length of anal body diameter-----	<i>dubius</i> , n. sp.	14
Tail three times anal body diameter-----	<i>hartingii</i> De Man (4), 1884	15

KEY TO THE GENUS *NYGOLAIMUS*—Continued

Pharynx with area bearing minute denticles:	Subgenera and species	Figure No.
Type <i>N. denticulatus</i> Cobb (2), 1922-----	<i>NYGOLAIMIUM</i>	
Length under 3 mm-----	<i>menzeli</i> Micol. (5), 1925.	16
Length 4 mm. or more-----	<i>denticulatus</i> Cobb (2), 1922	17

TECHNICAL DESCRIPTION OF SPECIES

Nygolaimus vulgaris, n. sp. (Fig. 1.)

Diagnosis:⁴

			12.0	12 0	
3.0	8 0	29.0	52.0	98.1	
1.2	2.1	2.5	2.7	1.5	1.1-1.8 mm.

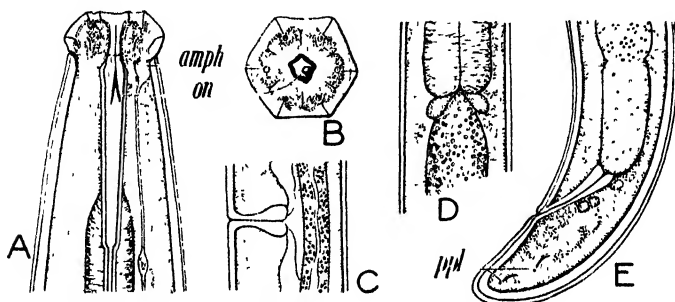


FIGURE 1.—*Nygolaimus vulgaris*, n. sp. A, Head, slightly subdorsal view; *amph*, amphid, *on*, tooth, B, face view showing arrangement of head papillae, also cross section of tooth and third section of pharynx, C, vulvar region, D, cardiac region, E, tail, *ppt*, caudal papillae

Cuticle with a distinct outer layer which is especially conspicuous on the tail. Spear very slender, three-fourths as long as the head width and hollow only one-third its length. Ovaries reflexed four-fifths their length. Males unknown from among several thousand specimens collected in all months of the year.

General characters.—Body assuming the form of an open letter C, especially when killed by gradual heat. Transverse striae indistinct. External amphids half as wide as the head. Second and third sections of pharynx obscure. Posterior portion of esophagus one-half as wide as the neck. Intestine one-half as wide as the body, its cells containing minute, colorless granules. Lateral chords one-seventh as wide as body.

The most prevalent nygolaim of the Western States. Collected from cultivated valley soils of Utah, Idaho, California, Colorado, and Oregon. See Table 1 for depth distribution.

Nygolaimus bisexualis, n. sp. (Fig. 2.)

Diagnosis:

			10.0	10.0	
1.9	8.0	27.0	52.0	98.2	
0.8	1.8	2.0	2.2	1.3	1.5-2.0 mm.
2.0	9.6	27.0	M	98.3	
0.8	1.8	1.9	1.9	1.4	1.7 mm.

Cuticle without a distinct outer layer as in *N. vulgaris*. Striae resolvable into rows of excessively minute dots. Spear very slender, as long as the width of the lips. Lateral chords one-fifth to one-third as wide as the body. Cardiac glands large and conspicuous. Ovaries reflexed only half their length. Males about equal in number to females.

⁴ The "Diagnosis" includes the formula and the first paragraph following. The remaining paragraphs give general, not necessarily specific, information.

General characters.—Intestine less than half as wide as the body, its cells filled with many dark granules that frequently give a slightly tessellated appearance. The last two pores of the lateral series usually are easily seen on the tail, but the remainder of the series are indistinct. Both sexes assume the form of an open letter C when killed by heat.

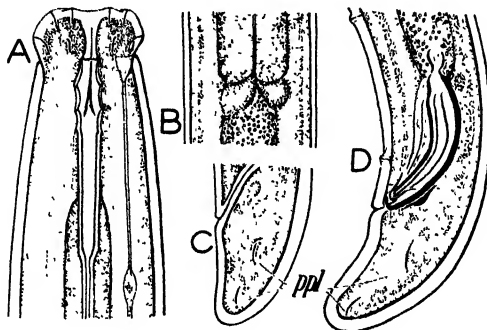


FIGURE 2.—*Nygolaimus bisexualis*, n. sp.: A, Head; B, cardiac region; C, female tail, *ppl*, caudal papillae; D, male tail

Spicula slightly arcuate with lateral guiding pieces and resting upon small gubernacula. A pair of papillae lie side by side just in front of the anus. About one and one-half body widths in front of these there usually is a ventral supplement.

Quite common in soils from canyons near Salt Lake City, Utah.

Nygolaimus tenuis, n. sp. (Fig. 3.)

Diagnosis:

			10.0	10.0		
1.7	6.0	22.0	52.0	98.9		
0.6	1.1	1.4	1.5	.9	2.0 mm.	

Spear only about half as long as the width of the lip region. Pharynx almost three times as deep as the width of the lips. Lateral chords only one-twelfth to one-tenth as wide as the body. Posterior portion of body not arcuate.

General characters.—The body twists more than in any other known nygolaim, frequently making half a turn or even more. Cardiac glands unusually large.

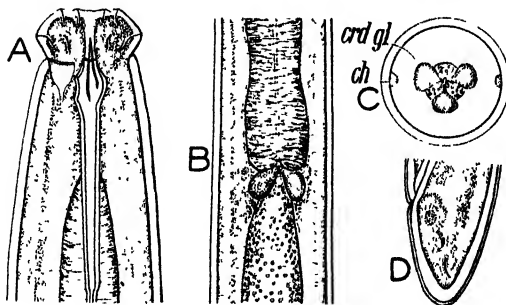


FIGURE 3 *Nygolaimus tenuis*, n. sp. A, Head, slightly sub-ventral view; B, cardiac region; C, cross section through cardiac glands, *crd gl*, cardiac glands, *ch*, lateral chord; D, tail

Intestine narrow and tapering at the anterior end except when gorged with food. Ovaries reflexed half their length. Rectum and prerectum each about as long as the tail. Males unknown among about 125 specimens.

Usually found in small numbers rather deep in sandy loam valley soils of Utah. See Table 1 for depth distribution.

***Nygolaimus amphigonicus*, n. sp.** (Fig. 4.)

Diagnosis:

			9.0	9.0		
1.8	7.5	23.0	54.0	98.5	1.9 mm.	
0.9	1.5	1.7	1.8	1.2		
1.7	8.0	26.0	M	98.1	1.53 mm.	
0.9	1.5	1.9	1.9	1.5		

Similar to *Nygolaimus tenuis*, but with these differences: Pharynx only about twice as deep as the width of the lips. Striae very fine but under favorable conditions are resolvable into dotlike markings. Lateral chords one-fifth to one-fourth as wide as the body. Males about as numerous as females.

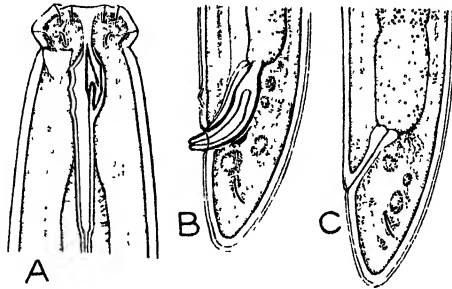


FIGURE 4—*Nygolaimus amphigonicus*, n. sp. A, Head, slightly submedial view, with tooth not yet completely joined to the pharyngeal wall after the last molt. B, male tail. C, female tail.

General characters.—Cardiac glands not conspicuously large. Both sexes assume an almost straight position when killed by gradual heat. Ovaries reflexed half their length, the front being on the right, the rear on the left, side of the body. Intestine three-fourths as wide as the body, its cells containing a few small, very dark, scattered granules.

From about roots of tomato plants, Buena Park, Calif., and mountain and desert soil from St. George, Zion Canyon, Salem, and Jensen, Utah.

***Nygolaimus pachydermatus* Cobb, 1915.** (Fig. 5.)

0.6	7.7	32.0	66.0	98.0	1.8 mm.
1.1	1.9	2.4	2.5	1.9	

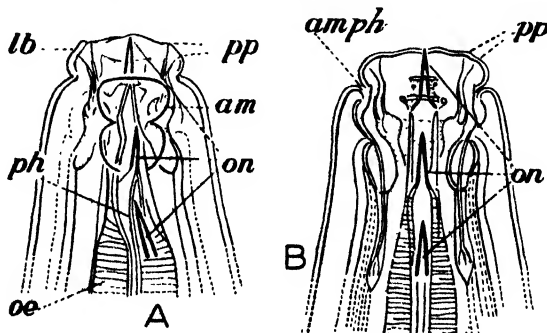


FIGURE 5—*Nygolaimus pachydermatus* Cobb: A, Head of molting specimen; *am*, amphid; *lb*, lips; *oe*, esophagus; *on*, tooth; *ph*, pharynx; *pp*, papillae; B, head, dorsoventral view; *amph*, amphid; *on*, tooth; *pp*, papillae. (After Cobb)

Measurements derived from a young specimen. Cuticle thicker on the tail. Lips connate, set off by constriction. Amphids half as wide as the corresponding

part of the head. Spear half as long as head is wide, conical, slender, pointed, toothlike; without guiding ring. Posterior half of the esophagus enlarged to three-fourths the width of the neck. Tail convex-conoid. Fixed in Flemming's solution and examined in glycerin. From the roots of cherry trees, Tokyo, Japan.

Nygolaimus obtusus, n. sp. (Fig. 6.)

Diagnosis:

			11.0	11.0	
1.8	7.5	30.0	60.0	98.6	2.9 mm.
0.7	1.8	2.2	2.2	1.6	

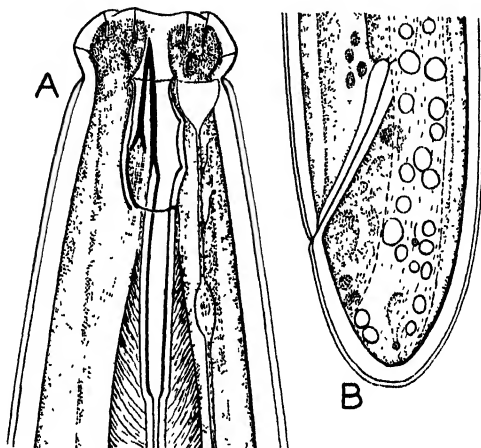


FIGURE 6—*Nygolaimus obtusus*, n. sp. A, Head, B, tail

Body but little curved, even in the posterior third. First three sections of pharynx forming a large cavity about one-third as wide as the head and twice as long as wide. Ovaries reflexed one-third their length. Tail, long hemispherical.

General characters.—Lateral chords one-fifth as wide as the body and conspicuous because of the large granules appearing in them. Cardiac glands unusually small. Intestine three-fourths as wide as the body, its cells densely packed with small granules. Anus depressed, rectum one and one-third times as long as the anal body diameter. Prerectum one and one-half times as long as the rectum. The elongate-hemispherical tail bears two pairs of conspicuous papillae, the posterior pair often showing distinct connections with an internal glandlike body. Amphids obscure, about one-third as wide as the head, with inner extensions leading to small sensillas. Behind the amphid and about opposite the base of the vestibule of the pharynx are located two pores with inner connections that apparently end in the tissues of the amphids.

No males found in the single collection of 12 specimens from a sugar-beet field near Ogden, Utah.

Nygolaimus brachyurus De Man. (Fig. 7.)

			9.0	9.0	
1.8	6.0	30.0	57.0	99.1	2.8–3.6 mm.
0.7	1.5	1.6	1.8	1.1	
1.7	6.8	29.5	M	98.9	2.3–2.9 mm.
0.7	1.6	1.8	1.8	1.1	

Several females of this species collected in Utah were sent to De Man, at Ierseke, Netherlands, to verify the determination. He made a careful comparison with the specimens that he had collected in the Netherlands and in Norway, and in a letter dated October 22, 1928, he says:

"The species seems to belong indeed to *Dorylaimus brachyurus* as described in my monograph of 1884. * * * In October, 1913, just 15 years ago, I had the good luck of discovering the male, at that time still unknown, of this species, in dry soil of a meadow in the western part of the Dutch province of North Brabant. This male was 1.55 mm. long, width 1.8%, esophagus 25.0%, tail 1.25%. Besides the usual anal papilla, still another papilla was observed in front of the anus, about twice as far distant from the anus as the length of the tail. Perhaps instead of one preanal papilla there are two submedian papillae placed abreast close by one another. * * * In August, 1918, a female was captured by me in soil covered with moss and grass from a wood in western North Brabant. The length was 3.72 mm., width 2.0%, esophagus 28%, tail 0.9%, vulva at 56%, front ovary 11.3%, rear ovary 11.6%. Another female 4.1 mm. in length was 1.75% wide, esophagus 25%, and tail 0.9%. There were two eggs in each of these females."

From this letter it is evident that the European and American specimens are practically identical in every respect and the species is without doubt a cosmopolitan one

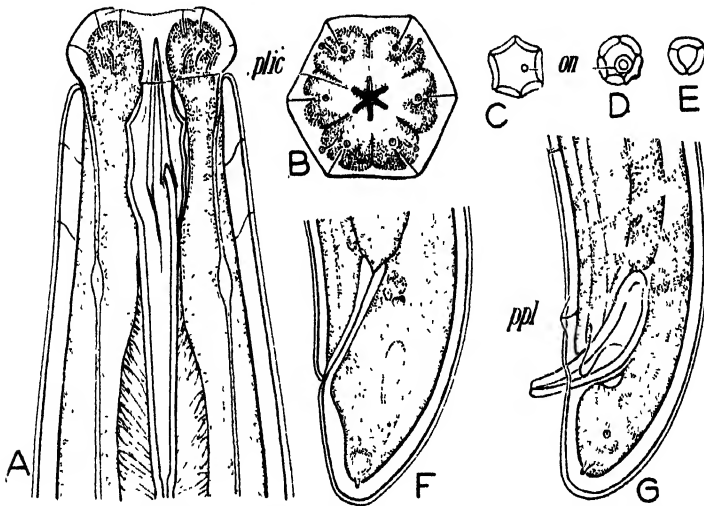


FIGURE 7. *Nyggolaimus brachyurus* De Man. A, Head, *plic*, plications of pharynx, B, face view, C, cross section of pharynx through second portion; *on*, tooth, D, cross section through third portion, E, same through fourth part, F, female tail, G, male tail, *ppl*, preanal papillae

Commenting on the tooth of this species, De Man says: "Spear comparatively feeble; it presented another structure than in other species and seemed to be placed in a chitinous case or tube."

Ten papillae in the outer circle. (Fig. 7, B.) Lip region one-third to one-half as wide as the base of the neck. Tooth about as long as the width of the lip region. Lateral chords one-eighth to one-sixth as wide as the body. Ovaries reflexed one-half their length. Rectum and prerectum each somewhat longer than the anal body diameter. The esophagus of immature specimens frequently occupies from 35 to 40 per cent of the body length.

In the Western States the males are rare—only about 1 to 50 females. The strong, slightly arcuate spicula rest on small gubernacula and possess lateral guiding pieces. (Fig. 7, G.) A pair of preanal papillae lie just in front of the anus, and two tail lengths in front of these is a rudimentary ventral supplement; occasionally a second is present one tail length in front of the first.

Small numbers of this species are generally distributed in desert, foothill, and mountain soil of Utah, Nevada, Idaho, and Oregon.

Nygotaimus aquaticus, n. sp. (Figs. 8 and 9.)*Diagnosis:*

			14.0	14.0	
2.1	6.0	34.5	51.0	98.5	2.5—3.7 mm.
0.8	1.9	2.5	2.7	1.5	
2.0	6.4	25.4	M	98.4	2.9 mm.
0.9	1.8	2.4	2.4	1.6	

Pharynx four times as deep as the width of the lip region. Tails of both sexes blunt and rounded, slightly longer than the anal body diameter. Female pre-rectum two and one-half times as long as the rectum.

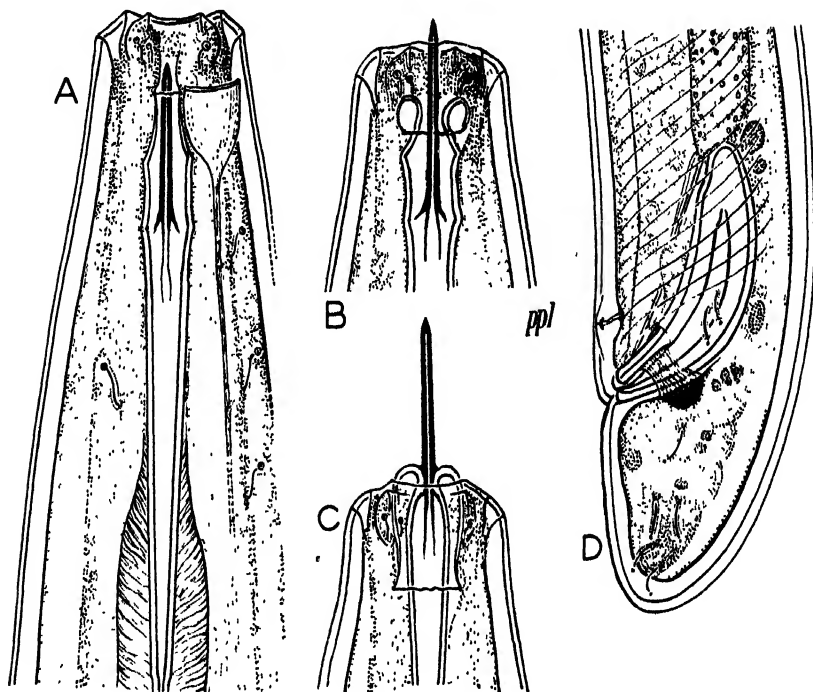


FIGURE 8.—*Nygotaimus aquaticus*, n. sp. A, Head, slightly submedian view with tooth in place in pharynx; B, same with tooth thrust partly forward and showing the manner in which the pharynx is everted; C, pharynx completely everted; D, male tail, *ppl*, preanal papillae

GENERAL CHARACTERS.—Ten papillae in the outer circle. Amphids half as wide as the head. Body pores distinct. Enlarged portion of the esophagus usually irregular in width. Cells of the intestine filled with small, brown granules. Rectum as long as the anal body diameter. Ovaries reflexed one-half their length.

Male tail bearing five to eight irregularly spaced, ventral, preanal supplements in addition to the pair of anal papillae. The slightly arcuate spicula are unusually large, rest on a small, somewhat hemispherical gubernaculum, and have furcate, lateral guiding pieces.

Collected from Utah Lake at an elevation of 4,750 feet, and from mountain lakes of Utah at elevations of 8,000 to 10,000 feet. Setae of oligochaetes of the family Naididae were found in several specimens.

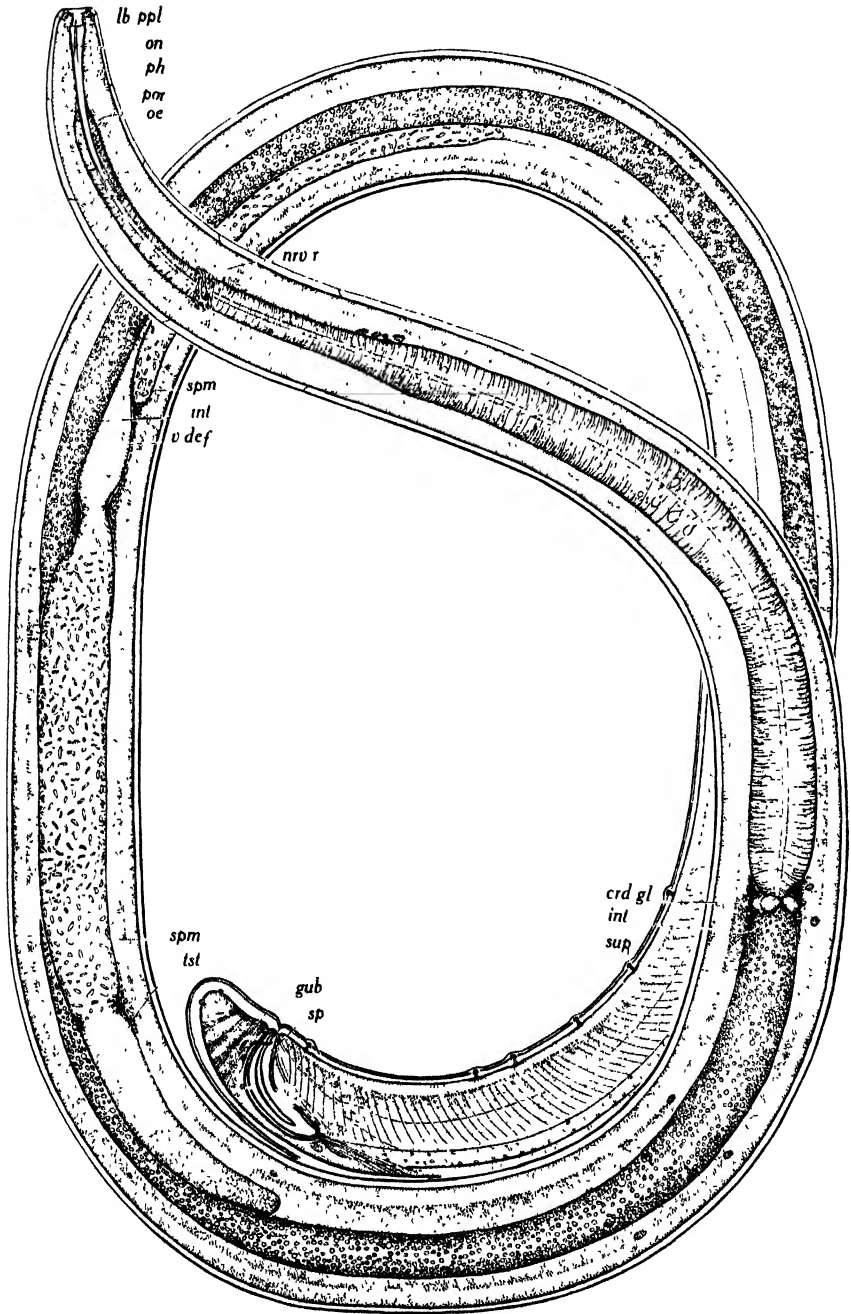


FIGURE 9.—*Nygolaimus aquaticus*, n. sp.: Male; *crd gl*, cardiac glands, *gub*, gubernaculum; *int*, intestine; *lb, ppl*, labial papillae, *nrv r*, nerve ring; *oe*, esophagus; *on*, tooth; *ph*, pharynx; *por*, lateral pore; *sp*, spicula; *spm*, spermatozoa; *sup*, supplements; *tst*, testes; *v def*, vas deferens

Nygalaimus ferox, n. sp. (Fig. 10.)*Diagnosis:*

			12.0	12.0	
1.8	5.0	15.0	46.0	99.5	
0.6	1.2	1.6	1.9	0.7	3.32 mm.
1.5	4.7	19.0	M	99.0	
0.5	1.5	1.7	1.7	1.0	4.3 mm.

Female tail slightly longer than half the anal body diameter. Esophagus less than 20 per cent of body length. Tooth one-third or one-half as long as the width of the lip region. Gubernacula thin and flat.

General characters.—Ten papillae in the outer circle. Amphids one-half as wide as the head, conspicuous from a dorsoventral view. Pharynx three times as deep as the width of the lip region. Posterior portion of esophagus half as wide as the neck. Prærectum and rectum of female both slightly longer than the anal body diameter.

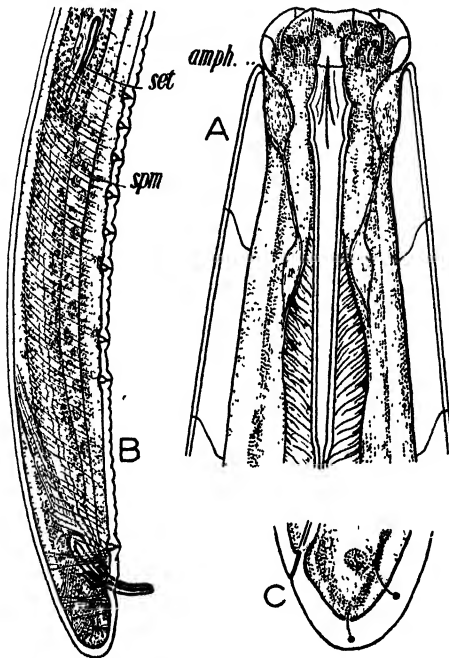


FIGURE 10.—*Nygalaimus ferox*, n. sp.: A, Head, dorso-ventral view; *amph.*, amphid; B, male tail, $\times 200$; *set*, seta of oligochaete; *spm*, spermatodon, C, female tail, $\times 350$

Male tail as long as the anal body diameter. The usual pair of papillae lie near the anus, and three tail lengths in front of it are eight irregularly spaced ventral supplements. The thick cuticle between them is marked by creases that give it a wrinkled appearance. The tooth of the male is one-third longer than that of the females in the collection.

Three females and one male from a stream bank, Alsee Mountain, near Corvallis, Oreg. The intestines were gorged with the remains of oligochaetes, including many setae and large pieces of cuticle.

Nygolaimus shadini Filipjev, 1928. (Fig. 11.)

1.6	5.4	20.0	53.0	98.6	3.0-3.71 mm.
0.6	1.7	1.9	2.0	1.1	
1.7	6.2	21.0	M	98.7	3.0-3.67 mm.
0.7	1.5	1.8	1.8	1.1	

Head continuous with body contour. Ten papillae in the outer circle. Tooth shorter than lip width. Posterior portion of esophagus almost filling the body cavity. Anal muscles conspicuous.

Spicula strong, broad, and arcuate, resting upon a little gubernaculum. Tail with two submedian papillae. A pair of papillae lie just in front of the anus,

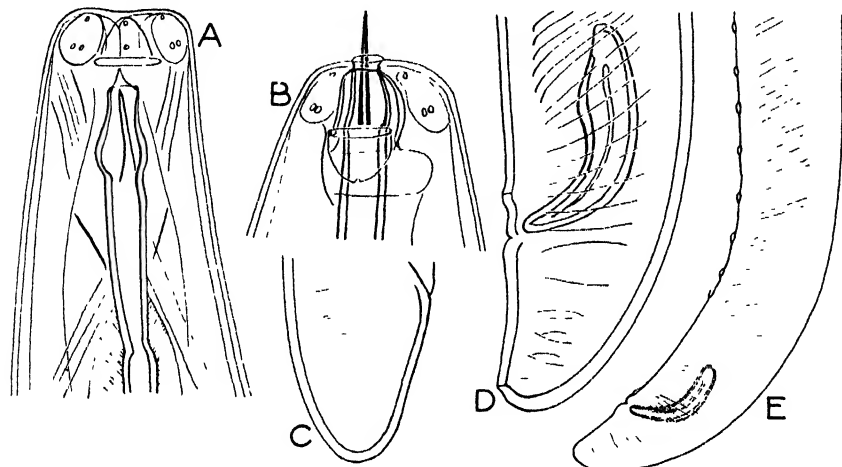


FIGURE 11 -- *Nygolaimus shadini* Filipjev. A, Head, B, lip region with tooth thrust forward, C, female tail; D, male tail, E, posterior portion of male. (After Filipjev)

and about two tail lengths in front of these begins a series of five to seven ventral supplements.

Four males and two females from mud, sand, and sphagnum, collected on the coast at mouth of Klazma River, Oka, District Baliff, Russia (3).

Nygolaimus teres, n. sp. (Fig. 12)*Diagnosis:*

			51.0			
2.5	8.0	23.0	M	98.6	1.62 mm.	
1.0	1.8	2.1	2.5	1.8		
			15.0	15.0		
2.5	8.0	23.0	50.0	98.4	1.66 mm.	
0.8	1.6	2.3	2.6	2.0		

Striae coarser than on any other nygolaim examined and usually can easily be resolved into dotlike markings. Tooth as long as the width of the lip region, hollow almost the entire length. Pharynx three times as deep as the width of the lips. Tail very blunt and rounded. Male with 6 to 10 ventral, preanal supplements in addition to the anal papillae.

General characters.—Lateral chords one-fifth as wide as the body. Ovaries reflexed one-half their length. Prerectum twice as long as the rectum. Lateral series of pores usually visible almost the entire length of the body. A pair of dorsal glands crowding the anterior end of the intestine usually is visible. These

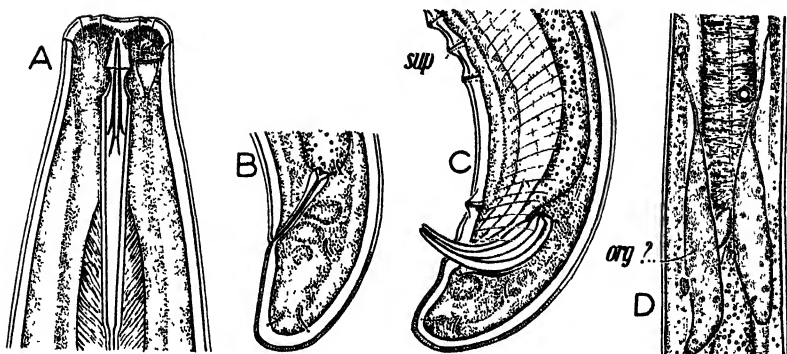


FIGURE 12—*Nygolaimus teres*, n. sp.: A, Head; B, female tail; C, male tail; *sup*, male supplements; D, cardiac region; *org*, organs of unknown function with their ampullae apparently connecting with cells in the lateral chords

vary greatly in size in different specimens. Males and females about equal in numbers.

Common in foothill and mountain soil in the Wasatch and Uinta Mountains, Utah

***Nygolaimus acuticaudatus*, n. sp.** (Fig. 13.)

Diagnosis.

		11.0	14.0		
1.9	6.4	20.0	48.0	97.8	
1.0	2.0	2.0	2.4	1.6	1.47 mm.
		60.0	M	97.6	
1.9	6.8	20.7			
1.0	1.6	2.0	2.2	2.0	1.47 mm.

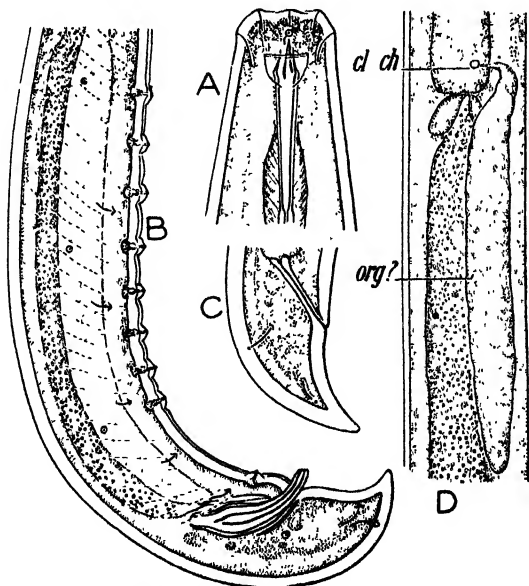


FIGURE 13—*Nygolaimus acuticaudatus*, n. sp.: A, Head; B, male tail; C, female tail; D, cardiac region; *cl ch*, cell in lateral chord; *org?*, organ of unknown function

Lip region continuous with the body contour. Tooth less than half as long as the width of the lip region. Pharynx about twice as deep as the width of the

lips. Tail but little longer than the anal body diameter, ending in a sharp terminus.

General characters.—Amphids obscure slits half as wide as the head. Posterior half of esophagus enlarged. The glandular organs of the cardiac region vary greatly in size, in some specimens being difficult to see, in others attaining a length of two or three body widths (Fig. 13, D.) Ovaries reflexed one-half their length. Rectum about as long as the anal body diameter. Prerectum three times as long as the rectum.

Male tail bearing a pair of papillae just in front of the anus, and beginning a little more than one body width in front of this pair is a series of 8 to 10 ventral supplements. There are seven pairs of submedian pores. The slightly arcuate spicula rest on slender gubernacula.

A rare species from high mountain soils. This may be the species described by Steiner (6) as *Dorylaimus lugdunensis*.

***Nygolaimus dubius*, n. sp. (Fig. 14.)**

Diagnosis.

			12.0	12.0	
3.0	9.0	24.0	46.0	96.8	
1.0	2.1	2.7	2.9	1.6	1.1 mm.

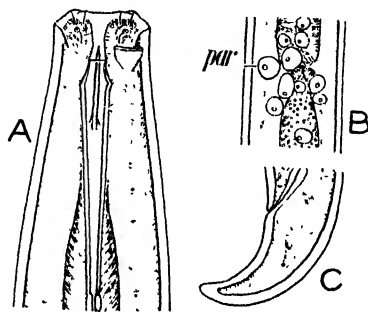


FIGURE 14. *Nygolaimus dubius*, n. sp. A, Head, B, cardiac region, par. parasitic sporozoans in body cavity, C, tail

Lip region continuous with the body contour. Tooth as long and pharynx three times as deep as the width of the lips. Tail ventrally bent, subacute, and twice as long as the anal body diameter.

General characters.—Posterior half of esophagus enlarged. Cardiac glands and organs crowding the intestine as in *N. hartingii*. Ovaries reflexed two-thirds to four-fifths their length. Rectum as long as the anal body diameter; prerectum two or three times as long as the rectum. No males found among about 40 specimens.

A comparatively rare species from the Wasatch and Uinta Mountains, Utah, at elevations of 10,000 to 12,000 feet.

This species may be identical with *Dorylaimus graciloides* Steiner (6).

***Nygolaimus hartingii* De Man, 1884. (Fig. 15.)**

			10.0	10.0	
3.0	7.5	22.0	45.0	95.6	
0.9	2.2	2.4	2.6	1.3	1.3 mm.

Specimens of this species collected in Utah were sent to De Man, and in his letter of October 22, 1928, he states that they apparently are identical with his *Dorylaimus hartingii*. The European specimens are slightly larger, 1.4 to 1.8 mm., but the body proportions are quite similar.

The conoid, subacute, ventrally bent tail is the most distinguishing feature of this species. Head continuous with the body contour. Tooth about as long and pharynx three times as deep as the lip width. Posterior half of the esophagus enlarged but irregular in size. The cardiac glands and organs crowding the intestines are conspicuous, and were figured by De Man in his original description. Ovaries symmetrical, generally reflexed about three-fourths their length. The position of the ovary in the body seems to vary. In one collection

of 22 specimens there were 20 with the front ovary on the left side of the body. In another group of 16 specimens from high mountain soil there were 11 with

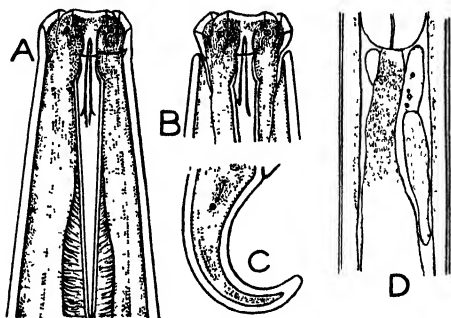


FIGURE 15 - *Nygolaimus hartingii* De Man. A, Head, slightly submedian view, B, hp region, dorso-ventral view, C, tail, D, cardiac region (After De Man)

the front ovary on the left side. Prerectum two to three times as long as the rectum. Males unknown from about 80 specimens.

Collected in a sugar-beet field adjacent to Utah Lake, near Provo, Utah, and at an elevation of 10,500 feet in Bells Canyon, near Draper, Utah.

Nygolaimus menzeli Micoletzky, 1925. (Fig. 16.)

3.5	?	30.0	51.0	98.8	
0.7	?	3.3	3.5	2.0	2.7 mm.

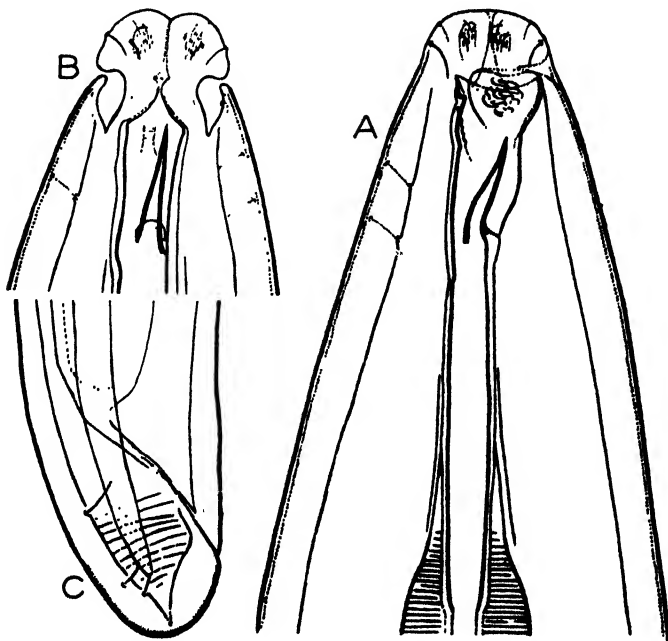


FIGURE 16.---*Nygolaimus menzeli* Micoletzky. A, Head, lateral view; B, head, dorso-ventral view; C, tail. (After Micoletzky (5))

Cuticle reaching a thickness of $6\ \mu$ near the head, $4\text{--}4\frac{1}{2}\ \mu$ at the middle, and $13\text{--}16\ \mu$ on the tail. Minute striations on the cuticle at the extremities. Lateral

chords one-seventh the body width. Innervated papillae on the tail and near the head. Anterior end of nema very considerably narrowed. Amphids beaker-shaped with many fibrils. Lips and papillae rudimentary. Pharynx over four times as deep as the width of the lip region. Esophagus at first one-fourth as wide as the body but later expanding to one-half to two-thirds as wide. Ovaries typical; vulva with strong sphincter and radial muscles; vagina reaching half-way across the body.

From Surinam, Dutch Guiana, South America

Nygolaimus denticulatus Cobb, 1922 (Fig. 17.)

1.8	5.4	22.0	—	Y	98.8	
1.1	1.3	1.6	—	1.6	1.4	4.3 mm.

Cuticle thick, transparent, colorless, naked, measuring $5\ \mu$ through near the head and $15\ \mu$ on the tail. Transverse striae very fine, barely resolvable into rows of excessively faint dots. Extending from near the head to the tail, there is a double series of about 150 sublateral pores not forming exact lines but arranged slightly irregularly. From each pore a narrow tube, $0.75\ \mu$ in diameter,

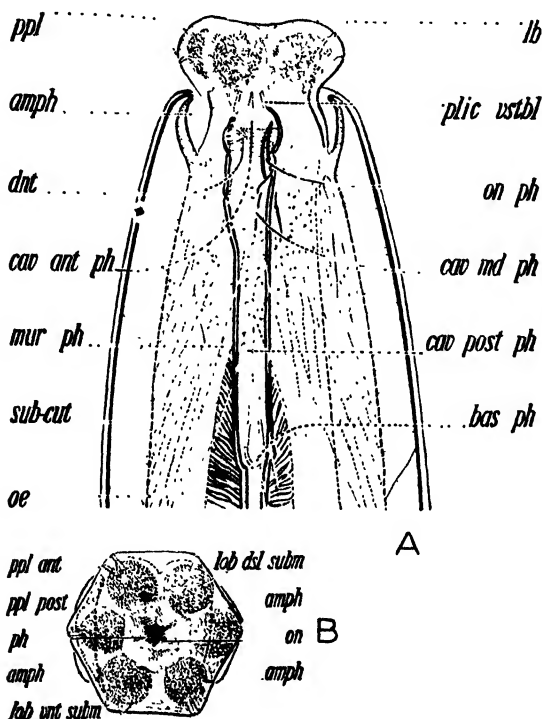


FIGURE 17. *Nygolaimus denticulatus* Cobb. A, Head, dorso-ventral view, *amph*, amphid, *bas ph*, base of pharynx, *cav ant ph*, cavity of anterior pharynx, *cav md ph*, cavity of median pharynx, *cav post ph*, cavity of posterior pharynx, *dnt*, denticles, *lb*, lips, *mur ph*, pharynx wall, *oe*, esophagus, *on ph*, tooth on pharynx wall, *ppl*, labial papillae, *sub-cut*, subcuticle. B, face view, *amph*, amphid, *lob dsl subm*, dorsal submedian lobe of lip region, *lob ant subm*, ventral submedian lobe of lip region; *ppl ant*, papillae of anterior circle, *ppl post*, papillae of posterior circle. (After Cobb (2))

extends to the lateral chord, where it rather suddenly expands and connects with a broadly saccate unicellular gland located in the tissue of the chord. The somewhat hexagonal lip region is distinctly set off by a constriction, and especially laterally, on account of the large and conspicuous amphids. The very

small left ventrally submedian toothlike onchium is only $6\ \mu$ long and $1\frac{1}{2}\ \mu$ wide at the base. The second portion of the pharynx is lined throughout the greater portion of its length with a multitude of excessively fine denticles, apparently similar to those found in the pharynx of some mononchs, and, as there, slightly larger and more regular anteriorly. The entire pharynx is nearly $60\ \mu$ long. Prerectum about five times as long as the corresponding width of the body. Rectum about as long as the anal body diameter. Nothing is known concerning the sexual organs.

Found in soil by J. R. Christie at Falls Church, Va., August 29, 1922. Only one young specimen seen. Examined and measured alive in water, and afterwards fixed in Flemming's solution and examined in glycerin.

SECTONEMA

TECHNICAL DESCRIPTION

Sectonema, n. g.

Diagnosis. Characters of *Dorylaimus*, with these differences: Tooth located in a ventral niche of the pharynx, grooved dorsally, and used with a slashing movement. Vestibule elongate. Gunding ring for tooth absent.

Type -- *Sectonema ventralis*, n. sp.

Sectonema ventralis, n. sp. (Fig. 18.)

Diagnosis. Characters of the genus and others as follows:

			14.0	14.0	
1.0	3.2	16.0	52.0	99.3	
0.3	.9	1.5	1.7	1.0	6.0 10 mm.

The thick cuticle is marked by fine crisscross lines similar to those found on certain Mermithidae (?). (Fig. 18, H.) The neck tapers to the narrow lip region, which is only one-fifth as wide as the base of the neck. The blunt rounded tail is almost hemispherical in form. The two series of lateral pores are a distinct feature, especially on the tail, where they are easily seen. The first pore is located just back of the amphid, and there is but a single line until near the base of the neck, where there is a divergence into two lines, which continue to the terminus of the body. Near the head are dorsal and ventral pores. The six distinct lips are set off by a deep constriction. There are 6 papillae in the inner and 10 in the outer circle. From a face view the vestibule and mouth opening are both found to be elongate. (Fig. 18, C, D.) The tooth is one-third to one-half as long as the width of the lip region and is set ventrally in the pharynx, reminding one somewhat of the tooth of *Nygolaimus*. It is grooved dorsally, and at its base the sides of the groove diverge to join the walls of the pharynx.

In cross section the pharynx is triquetrous. (Fig. 18, E.) It is composed of three sections arranged tandem. The first, thin walled and about as long as the tooth, forms the eversible portion. The second portion is about as long as the first, thickly cuticularized and forming a strong base for the attachment of the tooth. Dorsally this second portion does not appear to be differentiated from the third. The third part is three times as long as the second and narrows to join the lining of the esophagus.

When the nema attacks its prey the tooth is thrust out and the body of the victim is cut by a dorsal to ventral slash of the tooth. This slashing movement of the tooth apparently is accomplished by the use of the onchial muscle. (Fig. 18, A, *msc on*.)

The posterior three-fourths of the esophagus is enlarged by a gradual expansion. At the point where this expansion takes place there are three conspicuous pores in the lining of the esophagus, one dorsal, the other two subventral. The intestine is two-thirds as wide as the body, thick walled, and about 12 cells in circumference. It is almost always gorged with the remains of enchytraeids, which apparently form the principal food of this voracious species. Rectum as long as the anal body diameter, prerectum three to five times as long as the rectum.

Ovaries symmetrical and reflexed two-thirds their length, the front being on the left, the rear on the right side of the body. Eggs about as long as the vulva body diameter; as many as eight may occur in the body at one time.

This is one of the largest free-living nemas found in the Western States. It closely resembles *Dorylaimus regius* De Man in all respects except those of the tooth and pharynx. Frequently it is found associated with *D. regius*, which, like it, also feeds on oligochaetes (?). No males have been observed among several hundred specimens. From cultivated fields of Utah, Idaho, Colorado, and California.

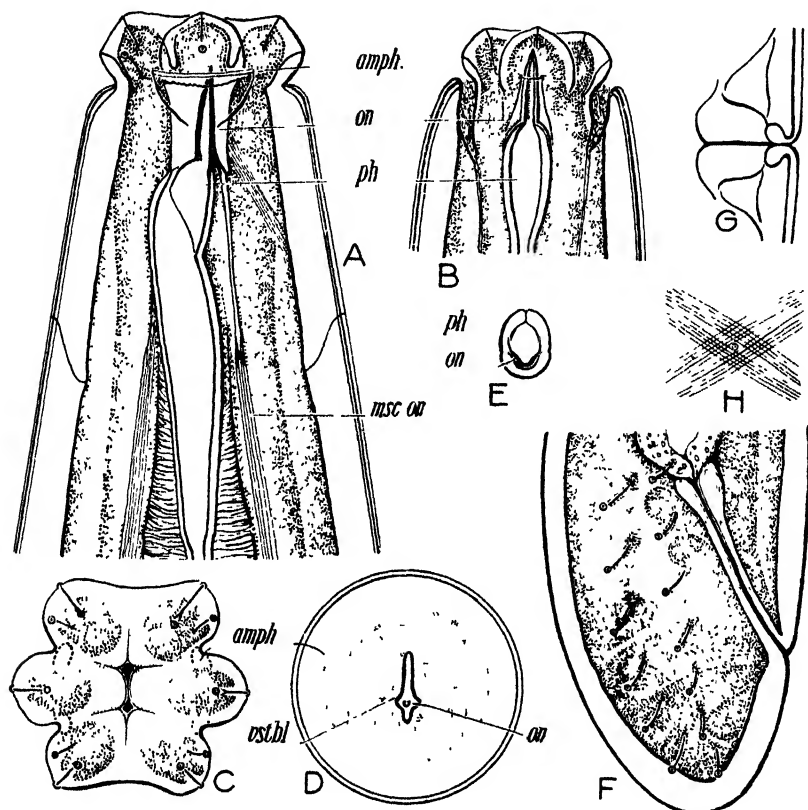


FIGURE 18.—*Sectonema ventralis*, n. g. n. sp. A, Head, lateral view, *amph*, amphid, *msc on*, muscle of tooth, *on*, tooth, *ph*, pharynx. B, lip region, dorso-ventral view. C, face view, *amph*, amphid. D, cross section through pharynx near base of tooth, *amph*, amphid, *on*, tooth, *vst bl*, vestibule. E, cross section of pharynx at base of tooth, *ph*, pharyngeal wall, F, tail, G, vulvar region, H, crisscross markings of cuticle showing one of the lateral pores magnified about 2,500 times

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A PRELIMINARY REPORT OF THE RELATION BETWEEN YIELD OF WINTER WHEAT AND MOISTURE IN THE SOIL AT SEEDING TIME¹

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INTRODUCTION

One of the problems that presents itself to the Kansas wheat farmer nearly every fall is whether conditions are such as to warrant seeding a large or a small acreage. Seasons in which the yields are not sufficient to return a profit, regardless of price or seed-bed preparation, are not uncommon. Obviously a reliable method of predicting yields would be of inestimable value, since acreage could be materially reduced in those years when prospects are poor and increased in others. As a matter of fact, farmers do habitually determine the acreage to be planted somewhat according to the prospects for a profitable yield based on the best available information. Thus in very dry falls or with ground in poor condition for other reasons a small acreage is likely to be seeded, whereas the opposite is true when moisture is plentiful and conditions are favorable for germination. However, the information on which such changes in acreage are made is very meager, indefinite, and often erroneous. For example, a good rain about seeding time may produce ideal topsoil conditions and thus encourage a large acreage without materially increasing the chances for a profitable yield if the subsoil is dry.

The studies on which this paper is based were undertaken to supply more accurate and dependable information regarding the relation between certain factors and the yield of the crop. It is a matter of common knowledge that moisture exerts a dominating influence on yields in western Kansas, and hence a study of factors influencing yields can not ignore this relationship. Moreover it would appear that moisture in the soil at seeding time especially merits attention (1) because of its possible relation to yield, and (2) because it can be determined accurately and without great expense.

That there is a relation between the moisture in the soil at seeding time and the yield of the following crop has been shown by Call and Hallsted.³ The utilization of soil moisture by spring wheat and the possible value of soil moisture as a factor in forecasting yields have been discussed by Cole and Mathews.⁴ The storage of water in soil and its utilization by spring wheat have been studied by Mathews,⁵

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² The authors desire to acknowledge the assistance of Prof. S. C. Salmon, of the Kansas Agricultural Experiment Station, especially in the statistical interpretation of the data.

³ CALL, L. E., and HALLSTED, A. L. THE RELATION OF MOISTURE TO YIELD OF WINTER WHEAT IN WESTERN KANSAS. Kans. Agr. Expt. Sta. Bul. 206, 34 p., illus. 1915.

⁴ COLE, J. S., and MATHEWS, O. R. USE OF WATER BY SPRING WHEAT ON THE GREAT PLAINS. U. S. Dept. Agr., Dept. Bul. 1004, 33 p., illus. 1923. (With an introduction by E. C. Chilcott.)

⁵ MATHEWS, O. R. STORAGE OF WATER IN SOIL AND ITS UTILIZATION BY SPRING WHEAT. U. S. Dept. Agr. Dept. Bul. 1139, 28 p., illus. 1923. (With introduction by E. C. Chilcott.)

and recently Alsberg and Griffing⁶ have emphasized the need of further work of this nature.

The data reported here were secured as a part of the investigations conducted by the Office of Dry Land Agriculture, United States Department of Agriculture, and the Kansas Agricultural Experiment Station cooperating at the Fort Hays branch station, Hays, Kans. The Fort Hays branch station is located at Hays, Ellis County, in west-central Kansas not far from the center of the Kansas hard red winter-wheat belt. The average precipitation recorded by the United States Weather Bureau for a 61-year period, 1868 to 1928, inclusive, is 22.8 inches. The average recorded by a rain gage on the project for the period during which these data were collected, namely, 1909 to 1928, inclusive, is 21.4 inches. Of this amount an average of 16.7 inches, or 78 per cent, fell during the growing season, April 1 to September 30, inclusive. The average evaporation from a free water surface during the growing season for the years 1909 to 1928, inclusive, is 46.7 inches.

The soil is a dark grayish-brown silty clay loam. The surface soil is 12 to 15 inches deep. It is a heavy soil as shown by its water relations, but its strong flocculation and granulation make the surface open and friable except when packed after heavy rains. It blows easily when the surface is smoothed by packing rains or by cultivation and after winter weathering.

The moisture-carrying capacity to a depth of 6 feet is about 22.6 per cent of the dry weight of the soil. The moisture-carrying capacity of the surface soil is slightly higher than that of the subsoil. The wilting coefficient is about 16.5 per cent. The minimum point of exhaustion for wheat as shown by observations is about 12.4 per cent.

The investigations at the Fort Hays branch station were begun in 1906 and had as their objective the study of tillage methods, rotations, and methods of conserving moisture for various crops grown in western Kansas. Soil-moisture studies in relation to wheat production have from the first taken a prominent part in these investigations, and they have been increased from time to time. The tillage and rotation project as a whole includes 461 tenth-acre plots, of which 383 relate to wheat production either directly or indirectly. More than 150 of the latter have been sampled for moisture at one time or another; about 50 individual plots comprising 483 plot years are included in the study presented here.

EXPERIMENTAL PROCEDURE

Soil samples for moisture determinations were taken at four locations on each plot, a tube cutting a core 20 mm. in diameter being used. The cores were extracted in 1-foot sections and placed in airtight metal containers. They were weighed in grams and milligrams and dried in an oven at a temperature ranging between 100° and 110° C. The moisture was calculated separately on each foot as a percentage of the dry weight of the soil.

Soil-moisture determinations have been made at various times during the period of seed-bed preparation and during the development of the crop, at all depths to and including 6 feet. Occasional deter-

⁶ ALSBERG, C. L., and GRIFFING, E. P. FORECASTING WHEAT YIELDS FROM THE WEATHER. Food Research Inst., Wheat Studies v. 5, no. 1, 44 p. 1928.

minations have been made to a depth of 10 feet. Casual studies of the data revealed a closer relation between yield and moisture in the soil at seeding time than between yield and moisture at any other time of the year. For this reason and because the purpose here is to derive information of use at or preceding the sowing of the crop, attention in this paper is confined to the moisture in the soil at or near seeding time, September 15 to October 1.

The method of study adopted was to make scatter diagrams showing the relation between moisture in the soil and yield and to calculate the coefficient of correlation between these two variables. For these purposes the percentage of moisture in the soil in the first, second, and third foot sections taken together and also in the fourth, fifth, and sixth foot sections taken together were correlated with yield. In some cases the correlation between yield and the moisture

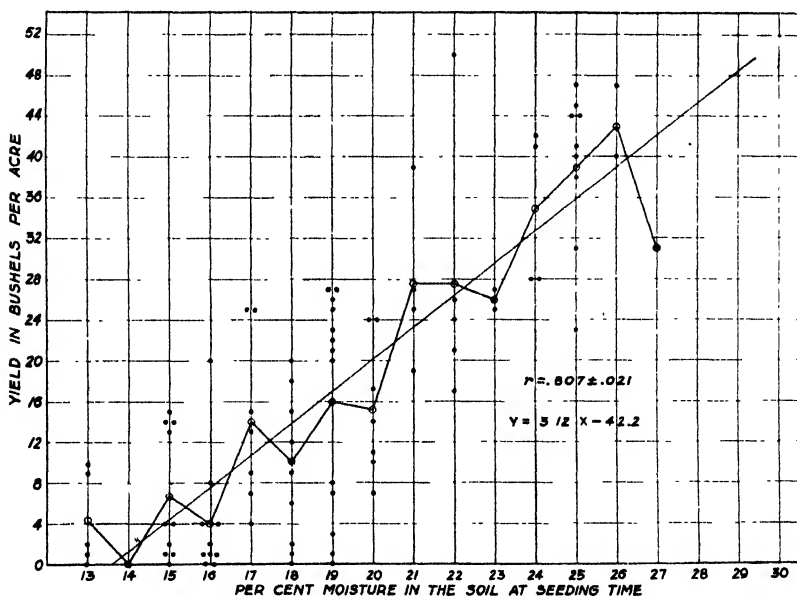


FIGURE 1 —Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following winter wheat

content of the full depth of 6 feet was calculated, but in all cases the figures so obtained were less useful for the present purpose than the others, and further consideration of these data is reserved for future study. The moisture determinations are reported to tenths of a per cent and the yields to tenths of a bushel. In calculating the correlation coefficients the decimals in both cases were disregarded, five-tenths and above being carried to the next whole number and decimals less than five-tenths being dropped.

Correlations were first calculated for single plots or for small groups of plots having identical or nearly identical treatment, and these were then grouped together where further study indicated that such grouping was justified. This might have been carried even further by combining some of these larger groups, but the data are not sufficient to indicate clearly in just what cases this might be done, and hence this possibility is reserved for future study.

EXPERIMENTAL DATA

Table 1 shows the previous crop and the treatment, the period of years covered, number of plot years, mean yield, mean moisture content of the first and the second 3-foot layers of soil, standard deviations of moisture and of yields, and the correlation between the moisture and the yield in each of the two soil layers. Table 2 gives the same information for larger groups, the basic data being the same in every case except for wheat following corn, in which case Table 2 contains some data that are not given in Table 1. Figures 1 to 6 are scatter diagrams showing the relations between the percentage of moisture in the surface 3 feet of soil at seeding time and the yields of wheat grown in the different cropping systems. Regression lines are shown in all cases except Figures 3 and 4, in which cases regression probably is not linear.

TABLE 1.—*Moisture content at seeding time, yield of wheat, standard deviations, and coefficients of correlation between soil-moisture content and yield of wheat grown in several cropping systems at the Fort Hays branch station, Hays, Kans.*

Group	Previous crop	Plot or rotation No. ^a	Period covered	Plot years		Mean yield per acre	Standard deviation of yield	Mean moisture in				Standard deviation of moisture in		Correlation coefficient for	
				Plot years				First, second, and third foot layers				First, second, and third foot layers		First, second, and third foot layers	
				No.	Bush	Bush	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
1	Wheat	A	1910-1928	17	9.5	9.7	16.6	14.5	3.8	1.3	0	862±0.042	0.308±0.148		
	do.	B	1910-1928	17	16.5	12.4	19.5	15.4	3.4	1.1		800±.059	418±.135		
	do.	E	1911-1928	16	18.8	13.9	19.9	15.6	3.4	1.1		807±.059	485±.104		
	do.	F	1911-1928	16	18.7	12.5	20.0	15.8	3.3	1.3		825±.054	606±.094		
	do.	401	1917-1928	9	21.8	17.1	19.8	17.8	2.4	1.4		742±.101	412±.198		
	do.	403	1917-1928	8	22.4	14.5	19.8	17.9	3.6	1.9		847±.067	148±.233		
2	Barley	404	1917-1928	9	19.7	15.2	19.0	16.8	3.3	2.3		960±.043	—	0.095±	223
	do.	403	1918-1928	8	25.4	15.0	20.9	18.3	3.0	2.1		778±.094	207±.228		
3	Corn	404	1917-1928	9	21.9	15.2	20.3	17.0	3.0	2.0		906±.040	—	0.313±	205
	do.	53	1911-1927	13	12.8	10.5	18.7	17.3	2.2	1.6		484±.126	768±.080		
4	do.	54	1911-1927	13	14.9	12.2	18.5	18.5	2.1	2.2		632±.098	220±.105		
	Kafir	349	1916-1928	11	11.5	9.2	15.4	17.1	1.9	1.3		326±.182	495±.154		
	do.	350	1916-1928	11	15.3	11.8	17.1	18.1	2.3	1.7		347±.179	194±.216		
5	Rye for green manure	51	1914-1921	7	17.4	9.1	20.4	16.6	2.2	1.6		763±.106	174±.247		
	do.														
	do.	55	1911-1927	15	15.8	10.0	20.3	17.2	2.3	2.3		568±.118	537±.133		
	Peas for green manure	56	1911-1927	15	16.6	11.6	20.1	18.2	3.0	3.2		618±.101	426±.184		
	do.														
	Fallow	92	1914-1921	7	18.7	10.2	19.6	16.9	2.6	1.6		485±.197	—	0.470±	255
6	CC C-D	1910-1928	17	26.4	13.8	24.0	19.8	1.6	2.1		442±.132	—	0.065±	168	
	MF H to S	1915-1922	46 ^b	24.2	12.7	22.9	20.4	2.4	3.0		416±.082	—	0.419±	082	
	57, 401, 402	1913-1928	30	22.7	12.4	23.4	19.0	2.3	2.3		562±.084	—	0.360±	107	
	501 to 510,	1911-1921	100	23.5	12.5	22.9	19.7	2.4	2.7		471±.042	—	0.413±	044	
	551 to 560														

^a The cropping systems indicated in the third column are as follows. Group 1. Plots A, B, E, and F are continuously cropped to wheat, plot A is late fall plowed, B is early fall plowed, E is early fall plowed and subsoiled; and F is early fall listed. Plots 401, 403, and 404 are 4-year rotations including various crops. In these plots wheat follows wheat. Group 2: Nos. 403 and 404 are 4-year rotations in which wheat follows barley. Group 3: Nos. 53 and 54 are 4-year rotations in which wheat follows corn. Group 4: Nos. 349 and 350 are 2-year rotations in which wheat follows kafir. Group 5: Nos. 51 and 55 are 4-year rotations in which wheat follows rye turned under for green manure and 56 and 92 are 4-year rotations in which wheat follows Canada field peas turned under for green manure. Group 6: Plots CC C-1 and MF H to S inclusive, are plots alternately cropped and fallowed. Nos. 57, 401, and 402 are 4-year rotations in which wheat follows fallow. Nos. 501 to 510, inclusive, and 551 to 560, inclusive, are 3-year rotations in which wheat follows fallow.

^b 8 plot years.

^c 9 plot years.

^d 13 plot years.

^e 16 plot years.

^f See Group 6, footnote a.

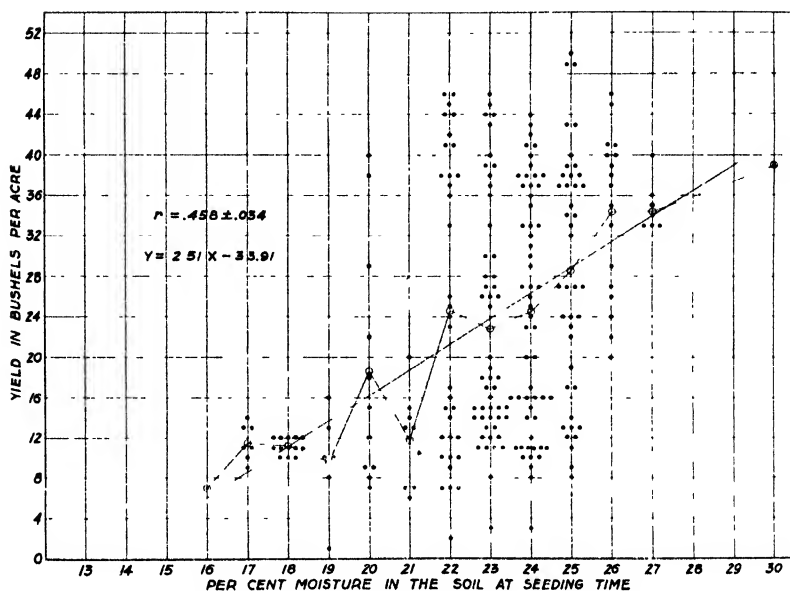


FIGURE 2 Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following fallow

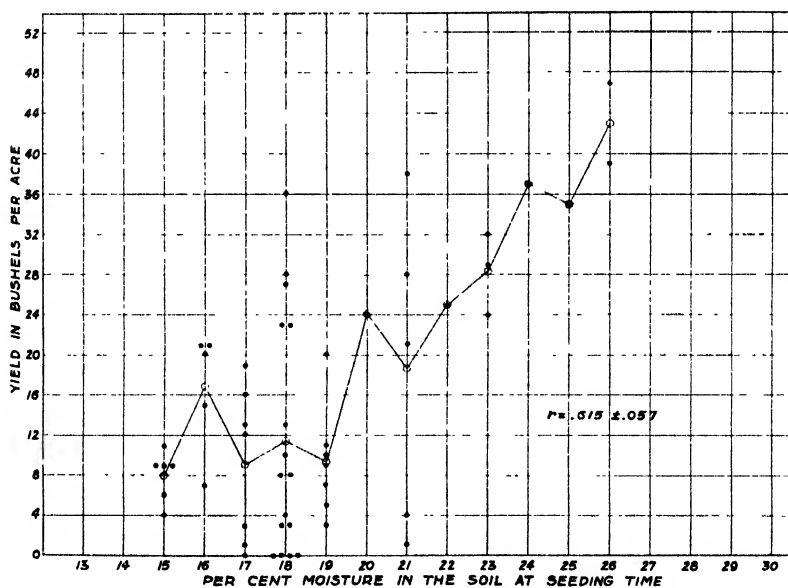


FIGURE 3.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following corn

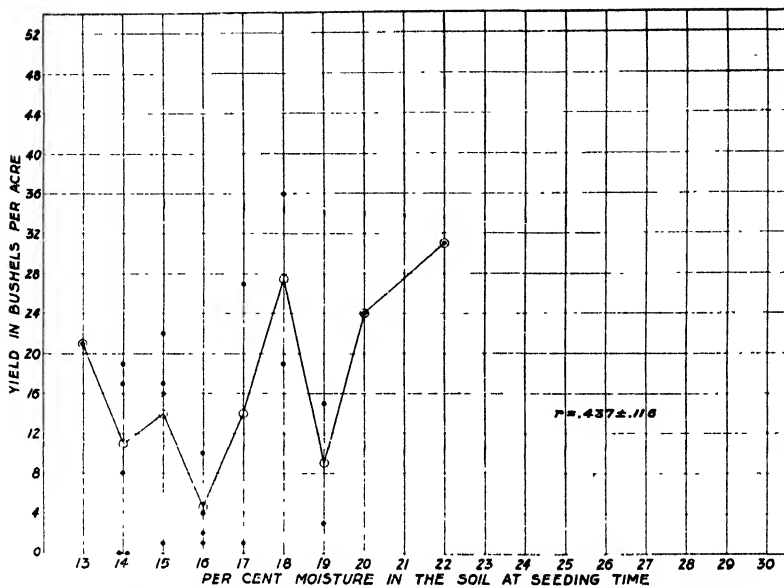


FIGURE 4.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following kafr

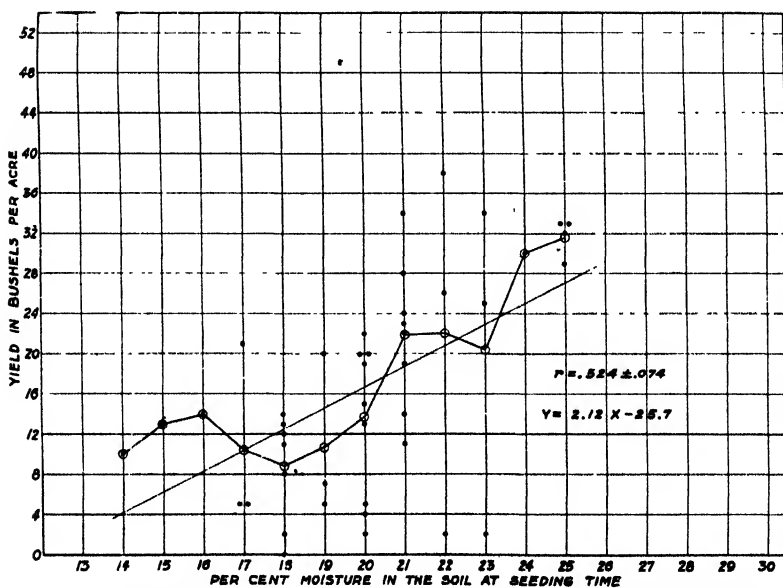


FIGURE 5 —Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following green manure

For all groups in Table 2, the correlations between moisture content of the first or surface 3 feet of soil and yield of wheat are significant in relation to the probable errors, showing an unmistakable relation between the two. There is likewise a significant correlation between yield and moisture in the second 3 feet for wheat after wheat, wheat after corn, and for wheat after fallow. In other cases the correlation is practically zero. In every instance but one the correlation with the moisture in the first 3 feet of soil is significantly higher than with the moisture in the second 3 feet. In this one exception, which was after fallow, the correlation for each section is relatively low.

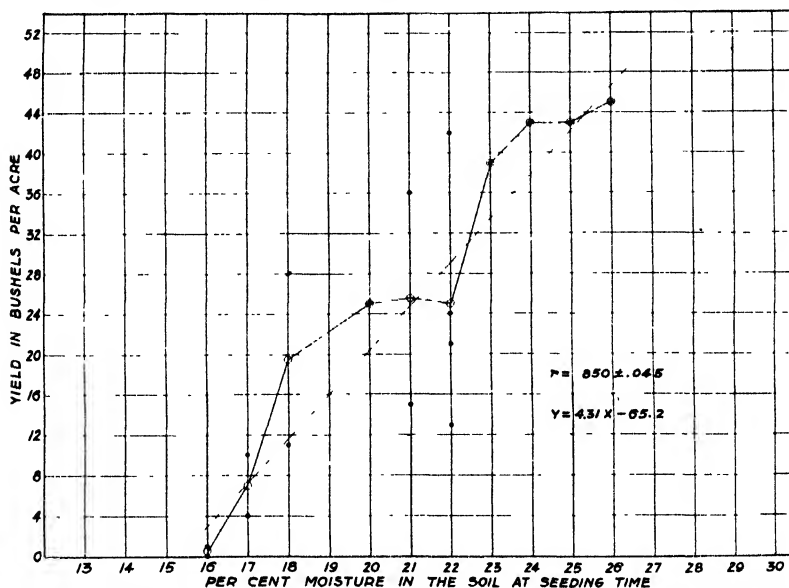


FIGURE 6.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following barley

TABLE 2.—The relation between moisture content in the soil at seeding time and yield of wheat grown in various cropping systems at the Fort Hays branch station
[Results arranged in larger groups]

Previous crop	Period covered	Plot years	Mean yield per acre	Standard deviation of yield	Mean moisture in —		Standard deviation of moisture for —		Correlation coefficient for —	
					First, second, and third foot layers	Fourth, fifth, and sixth foot layers	First, second, and third foot layers	Fourth, fifth, and sixth foot layers	First, second, and third foot layers	Fourth, fifth, and sixth foot layers
		Number	Bush	Bush	Per cent	Per cent	Per cent	Per cent		
Wheat.....	1910-1928	92	17.3	13.9	19.1	^a 15.9	3.6	^a 1.80	.807 ± 0.021	^a 0.402 ± 0.059
Barley.....	1917-1928	17	23.5	15.2	20.0	17.6	3.0	2.1	.850 ± .045	.07 ± .164
Corn.....	1911-1928	55	15.1	12.1	18.7	^b 17.8	2.8	^b 2.3	.615 ± .057	.342 ± .085
Kafr.....	1916-1928	22	13.4	10.7	16.2	^c 17.6	1.7	^c 1.6	.437 ± .110	.012 ± .144
Green manure.....	1911-1927	44	16.8	10.5	20.1	^d 17.4	2.6	^d 2.5	.524 ± .074	^d .145 ± .105
Fallow.....	1910-1928	253	23.8	12.6	23.0	^e 19.7	2.3	^e 2.7	.458 ± .034	^e .403 ± .036

^a 91 plot years.

^b 49 plot years.

^c 18 plot years.

^d 40 plot years.

^e 252 plot years.

A complete and critical study of the relation of soil moisture to yield should take into consideration the supply of moisture in the lower depths. Thus, it is conceivable that a correlation of 0.402 as was secured for the yield and moisture in the second 3-foot layer on wheat after wheat may be useful when considered in connection with the correlation in the upper 3 feet. The same applies to wheat on fallow and to wheat on corn ground. Some consideration has been given to these possibilities, but a preliminary study by means of multiple and partial correlations indicated that so far as the purpose of the present paper is concerned, no great improvements would be secured by the use of these data. The present report, therefore, is based on the first 3 feet of soil and the matter of the lower depths is reserved for future study. For the practical purpose of predicting yields, it is fortunate that the correlation between yield and moisture in the first 3 feet is relatively high, since the determinations for only 3 feet are much easier and more convenient to make.

The highest correlations were secured where wheat was grown after wheat or barley, the coefficients being 0.807 ± 0.021 and 0.850 ± 0.045 , respectively. The difference between them is less than the probable error of difference. Because of this fact and because of the small numbers involved in the latter case, no distinction between the two is made at this time.

The correlations for wheat after corn and after kafir are 0.615 ± 0.057 and 0.437 ± 0.116 , respectively. These lower values are in line with expectations in indicating that factors other than total moisture in the upper 3 feet of soil play an important part in determining the yield. A dry surface soil at seeding time may result in poor germination and a thin stand. A thin stand is usually accompanied by weed growth in the spring and late maturity, which subjects the crop to a longer period of danger of damage from hot winds, rust, and insects. Corn and kafir stubble lands are also more subject to soil blowing, which sometimes reduces yields. The well-known deleterious effect of sorghums on succeeding crops may also be a factor in reducing the yield of wheat after kafir. It should be noted, however, as previously mentioned and as illustrated in Figures 3 and 4, regression is probably not strictly linear in these cases, and hence the relation between moisture and yield may be closer than the calculated values of r suggest. In fact, it appears more than likely that the relation between soil moisture and yield when the moisture content of the soil is less than about 20 per cent is different than when it is 20 per cent or more. However, the available data are hardly sufficient for a critical discussion of these relationships.

The correlations where wheat is grown after green manure (0.524 ± 0.074) and after summer fallow (0.458 ± 0.034) are nearly the same, the difference being less than the probable error of difference. Wheat grown on land prepared by either of these methods sometimes suffers from the same causes, and the explanation for the relatively low correlation is probably the same for each. During the period here considered, there have been years when the rainfall was not enough or was too poorly distributed to finish properly the heavy growth produced on fallowed or green-manured plots. When such conditions prevail until after the stored moisture becomes exhausted, what promised to be a heavy yield may suddenly become an almost complete failure. Such conditions obtained in 1911, 1913, 1917, and 1927.

On the other hand, in seasons of high rainfall, such wheat makes a heavy growth and is more subject to injury from lodging. Under these conditions it often yields even less than cropped land. This was the case in 1915 and 1919.

A correlation of 0.8 or more, as was secured for wheat after wheat and wheat after barley, should be of value in predicting yields on the basis of moisture in the soil at seeding time. Consideration of the graphs (figs. 1 to 6) also will permit certain useful predictions regarding yield even when the correlation is considerably less than 0.8. For purposes of prediction the regression equation is useful, and consequently it has been calculated for each set of data and is shown in the figures. In two cases (to be noted later) the regression is not strictly linear. The same is perhaps true in other cases, although, in general, the relation is so nearly linear as to introduce no material error in considering it so. The numbers involved in general are too small to justify calculation of the correlation ratio.

Figure 1 shows a total of 92 comparisons of the relation between the water content at seeding time of land that has borne a crop of wheat and the yield of the following crop of wheat. In 35 of these the water content of the surface 3 feet averaged 20 per cent or more, which by the regression formula would indicate a probable yield of 20 bushels or more per acre. The yield was less than the indicated minimum in only 7 of the 35 cases. In only 1 of these 7 cases could the yield be characterized as very poor.

In 49 of the 92 comparisons the soil at seeding time contained 18.5 per cent of water or more, which would indicate a yield of 15 bushels or more. In only 9 of these 49 cases was the yield less than 15 bushels. In only 6 cases was the yield less than 10 bushels per acre.

TABLE 3. *Calculated and actual wheat yields when three different methods were used in preparing wheat stubble land for planting*

[$y = 3.12x - 12.24$]

Crop year	Late fall plowed			Early fall plowed			Early fall listed		
	Soil moisture	Yield per acre		Soil moisture	Yield per acre		Soil moisture	Yield per acre	
		Calculated	Actual		Calculated	Actual		Calculated	Actual
	Per cent	Bushels	Bushels	Per cent	Bushels	Bushels	Per cent	Bushels	Bushels
1910	22	26.4	20.5	24	32.6	27.8			
1911	14	1.4	0	19	17.0	3.3	18	13.9	0.6
1912	13	0.6	2.3	15	4.6	13.8	19	17.0	26.6
1913	16	7.7	8	16	7.7	2.3	16	7.7	8.4
1914	19	17.0	20.6	21	24.3	24.8	19	17.0	23.1
1915	13	0	9.3	15	4.6	13.1	15	4.6	13.9
1916	18	13.9	8.7	25	35.8	22.7	23	20.5	23.1
1917	13	0	0	18	13.9	2.0	16	7.7	0
1918	13	0	9.7	17	10.8	14.9	23	20.5	24.8
1919	(a)								
1920	19	17.0		20	20.2	24.4	22	26.4	24.2
1921	15	4.6	4.2	21	24.3	26.9	23	29.5	27.1
1922	13	0	5	17	10.8	6.8	17	10.8	13.3
1923	(b)								
1924	27	42.0	31.1	25	35.8	38.1	26	38.9	40.3
1925	16	7.7	0	16	7.7	1.2	17	10.8	3.7
1926	16	7.7	3.5	20	20.2	14.8	21	24.3	19.2
1927	15	4.6	3.5	18	13.9	6.3	20	20.2	6.8
1928	20	20.2	24.2	25	35.8	41.2	25	35.8	44.2

^a No moisture determinations near seeding time were made of 1919 crop

^b Crop of 1923 was destroyed by hail.

On the other hand, there are 26 cases where the moisture in the soil was 16.7 per cent or less, indicating a yield not exceeding 10 bushels. In only 6 cases was a yield of as much as 10 bushels obtained, and in only 1 of the 6 was the yield as much as 20 bushels per acre. In 18 of the 26 cases the yield was less than 5 bushels per acre.

It may be of interest to pursue this comparison somewhat further, and for this purpose Table 3 has been prepared. This table gives the moisture at seeding time in soils prepared by three different methods, the yield that would have been predicted by the regression equation, and the actual yield.

The correlation for moisture and yield on fallow was found to be only 0.458, hence any predictions of yield are certain to be less accurate than for wheat after wheat. Nevertheless, it appears that the relation is sufficiently close to be of value. Figure 2 shows that there were 226 cases out of a total of 253 in which there was 20 per cent of moisture in the soil at seeding time. According to the regression equation, a yield of 15 bushels or more should have been obtained. Actually this quantity (or more) was obtained in 165 cases, or in all but 60. Of these 60 cases, only 3 may be considered as complete failures (2 to 3 bushels per acre). Less than 15 bushels per acre would have been predicted in 27 cases. In only 1 of these cases was a yield of 15 bushels or more produced.

The moisture content of the soil at seeding time, the yields that would have been predicted from the regression equation, and the actual yields for three different plots of wheat grown on fallow are given in Table 4.

TABLE 4.—*Calculated and actual yields of wheat on fallow*

$$[y = 2.51x - 33.91]$$

Crop year	Soil moisture	Yield per acre		Soil moisture	Yield per acre		Soil moisture	Yield per acre	
		Calculated	Actual		Calculated	Actual		Calculated	Actual
		Per cent	Bushels		Per cent	Bushels		Per cent	Bushels
1910	25	28.8	42.5	(a)	—	—	(a)	—	—
1911	24	26.3	2.6	(a)	—	—	(a)	—	—
1912	20	16.3	20.2	(a)	—	—	(a)	—	—
1913	22	21.3	10.3	24	26.3	16.4	(a)	—	—
1914	26	31.4	21.6	21	26.3	19.9	25	28.8	26.8
1915	23	23.8	11.3	23	23.8	17.5	23	23.8	12.5
1916	27	33.9	33.8	25	28.8	31.8	26	31.4	37.8
1917	22	21.3	7.1	24	26.3	7.9	25	28.8	8.8
1918	23	23.8	35.8	18	11.3	9.8	19	13.8	7.9
1919	(a)	—	—	23	23.8	15.3	23	23.8	16.3
1920	25	28.8	37.0	27	33.9	33.0	25	28.8	26.7
1921	21	26.3	37.1	24	26.3	42.3	25	28.8	39.6
1922	24	26.3	25.2	23	23.8	32.5	21	26.3	24.3
1923	(b)	—	—	—	—	—	—	—	—
1924	25	31.4	45.6	(a)	—	—	(a)	—	—
1925	21	26.3	23.3	21	26.3	22.3	25	28.8	22.2
1926	21	26.3	26.2	21	18.8	19.7	21	18.8	12.8
1927	21	26.3	10.8	24	26.3	16.0	22	21	10.3
1928	25	28.8	48.7	25	28.8	49.0	25	28.8	50.0

a No moisture determinations were made near enough to seeding time to be of value.

b The 1923 crop was destroyed by hail.

Although the correlations are not high, 0.615 and 0.437, 20 per cent moisture in the upper 3 feet of soil on corn or kafir land has nearly always produced a crop, only 2 failures out of a total of 14 cases having been recorded. However, it is more difficult to store

as much as 20 per cent of moisture in the soil after these crops than after small-grain crops because corn and kafir mature several weeks later than the small grains and therefore afford a much shorter time in which to accumulate moisture before seeding time for wheat. Thus, of the 55 cases in which wheat has been grown after corn, there were only 14, or slightly more than 25 per cent, in which the moisture content of the upper 3 feet of soil was 20 per cent or more. Of the 22 cases of wheat after kafir (fig. 4), only 2 had 20 per cent or more.

With less than 20 per cent in the upper 3 feet of soil, the average yields are low and uncertain. Thus, for this group the average yield after corn is 10.9 bushels and on kafir ground 12 bushels.

DISCUSSION AND CONCLUSIONS

With reference to the practical side of the problem, it has already been pointed out that a moisture content of 20 per cent or more in the upper 3 feet of soil at seeding time at Hays has practically precluded a crop failure as a result of drought, although failure may result from hail, flood, or other conditions. This fact should be of considerable practical value, not only in discouraging a large acreage when the supply of moisture is materially less than 20 per cent, but also by way of emphasizing the need for those practices which will conserve the most moisture in the soil. In the period covered by these studies it has been possible by means of fallow to conserve as much as 20 per cent of moisture in the upper 3 feet of soil in nearly 90 per cent of the cases, for wheat after small grains in about 40 per cent of the cases, for wheat after corn in about 25 per cent of the cases, and for wheat after kafir in only about 10 per cent of the cases.

With a soil-moisture content of much less than 20 per cent in the surface 3 feet at seeding time the chances of securing a large crop are much less and the chances for harvesting a small crop or having a failure are measurably increased. With about 15 per cent moisture in the soil, the crop is practically dependent on rainfall subsequent to seeding.

While within certain limits it appears that the data presented here may be of considerable value for forecasting yields, they are principally useful in predicting failures, or more accurately, as a means of determining when chances for a crop are reasonably good and when they are largely dependent on factors other than stored moisture. The smaller the quantity of stored moisture at seeding time, the more dependent is the crop on the weather during the growing season and the greater are the chances of a failure.

COLOR OF POTATO CHIPS AS INFLUENCED BY STORAGE TEMPERATURES OF THE TUBERS AND OTHER FACTORS¹

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INTRODUCTION

In the fall of 1928 an inquiry was received at the Maine Agricultural Experiment Station from a manufacturer of potato chips in regard to the cause of dark color² in potato chips. He stated that frequently in the winter he had difficulty with northern-grown potatoes, because potato chips made from them were dark colored. A later survey showed that this has been the experience of other chip producers to the extent that some discriminate definitely against northern-grown potatoes. Furthermore, wholesale receivers and jobbers on the Boston market have reported that they have occasional complaints from proprietors of hotels and restaurants about the color of French-fried potatoes made from certain lots of these tubers.

Vosbury (9)³ made a study of some phases of potato-chip making and concluded that the troubles of manufacturers are generally due to the use of varieties not adapted to chip making, to improper washing of the sliced potatoes, or to the use of oil at too low temperatures for frying. She did not mention any outstanding differences in color. Woodruff and Blunt (10) state that they found that browning of chips varied in different fats, but their data do not give any clues as to the reasons for the darkening that commercial producers find associated with certain lots fried under their usually standardized conditions.

It was known that northern-grown potatoes would frequently be held in cool storage and that this increased their sugar content (1, 4). Since many foods brown during cooking as a result of the caramelization of the sugar which they contain, it was suspected that it was the accumulated sugar in the potatoes which caused chips made from them to be excessively dark.

PLAN OF EXPERIMENT

In some preliminary experiments, tubers stored in a refrigerator at 35° to 40° F. fried darker than those from the same lot held at room temperature. Consequently, a series of experiments was planned for the season of 1929 to ascertain the effect of cold storage on color of chips from various lots of potatoes. Since Appleman (1) and Hopkins (4) had also reported that sugar which had accumulated in the cold disappeared when the tubers were held at temperatures above 40° F., some of each lot were to be returned to warm storage for varying periods before frying.

¹ Received for publication May 23, 1930; issued October, 1930

² The word "dark" is used in this paper to refer to varying shades of brown which are darker values of the light brown or golden brown tints associated with desirable color in potato chips

³ Reference is made by number (italic) to Literature Cited, p. 490

SELECTION OF POTATOES

The potatoes used were grown on the experimental farms of the Maine Agricultural Experiment Station, that is, Aroostook farm at Presque Isle in the northeastern part of the State, and Highmoor farm at Monmouth in the south-central part.

The lots of tubers were divided into two series, series A, consisting of tubers from plots in which there were no recognized signs of disease or in which diseased plants were rogued out early in the season, and series B, consisting of potatoes from plants with known disease and from rows grown next to such diseased stock so that there was strong likelihood of infection.

Series A included: (1) Green Mountain potatoes from both farms and Bliss Triumph and Irish Cobbler tubers from Aroostook Farm; (2) a set of Green Mountain potatoes dug at different dates during the growing season to provide material for study of the effects of differences in maturity at digging; (3) a collection of Green Mountain hills bagged separately at digging; and (4) potatoes from two tuber-line strains of Green Mountain. The last three collections were grown at Highmoor farm.

Series B included Green Mountain tubers produced at both farms by plants showing, or by healthy plants near those showing, the following diseases: Spindle tuber, leaf roll, rugose mosaic, mild mosaic, and giant hill. Symptoms of these diseases as they appear in Maine have been described by Schultz (6) and by Schultz and Folsom (7).

All Green Mountain tubers were from plots supervised and rogued by Donald Folsom, plant pathologist at the Maine Agricultural Experiment Station.

CONDITIONS OF STORAGE

With the exception of those potatoes in series A dug at earlier dates to show the effects of immaturity, all were harvested late in September or early in October and shipped immediately to Orono. Here they were stored in a vegetable cellar where thermograph records showed that the temperature seldom went below 40° F., and then for only a few hours at a time. The upper limit of the range was around 55° F.

On October 18 eight samples of each of the above kind of potatoes were selected and placed separately in paper bags. These were regrouped to form eight lots which were handled as indicated later. The cold storage was commercial and was said to vary from 32 to 37° F. Warm storage was in rooms which varied from 60 to 70° F.

The experimental lots were held under the conditions outlined below:

Lot 1.—Fried immediately as control.

Lot 2.—Fried after two weeks in cold storage.

Lot 3.—Control, held in cellar and fried two weeks after lot 1.

Lot 4.—Fried after four weeks in cold storage.

Lot 5.—Fried after four weeks of cold storage followed by one week of warm storage.

Lot 6.—Fried after four weeks of cold storage followed by two weeks of warm storage.

Lot 7.—Control, held in cellar and fried seven weeks after lot 1.

Lot 8.—In cold storage four weeks. Then each sample of 10 tubers was divided, and one-half was fried after warm storage for four weeks and the remainder after warm storage for eight weeks.

EXPERIMENTAL METHODS

FRYING TECHNIC

It was of course necessary to standardize the frying technic to eliminate variations due to differences in temperature and in time of cooking. Unless otherwise stated, 10 tubers were taken as a sample. These were weighed and then measured with a sliding caliper so that it might be possible to correlate any differences with size and shape of the tuber.

Each tuber was divided transversely, approximately in the middle. One-half was thinly pared to about 1 centimeter back from the cut surface and two slices one-sixteenth of an inch (0.16 centimeters) in thickness were cut from the median surface with a vegetable slicer for frying. Tests showed that these represented the tuber adequately. All of the chips from one sample (10 potatoes) were washed in running water for the few minutes before the next sample was ready. Then they stood in pans of cold water until cooked. This period was usually about an hour.⁴ The slices were blotted dry with paper towels before being placed in the hot oil.

For the frying, a uniform amount (1,000 gm.) of a high-grade cottonseed oil was heated in a heavy, straight-sided aluminum pan at constant rate of heating on an electric hot plate. The pan was of such size that the oil was 6 centimeters deep. Ten chips, one from each potato in the sample, were dropped into the oil when the thermometer indicated a temperature of 400° F. They cooled the oil, sometimes to a temperature as low as 350° in the case of the larger chips, but the continuous heating brought the temperature quickly up to about 375°, and for most of the cooking period the range was from 375 to 400°. Tests showed that frying temperatures below 350° were required to alter the color of the chips produced.

At first a stop watch was used to time the frying period, but it was found that the time required to produce chips which were properly cooked depended too much upon the cooling action of the slices (which varied with their size) for exact comparisons. However, it was easy to fix as a definite end point the instant when the chips became opaque and bubbling ceased almost completely. This cessation of bubbling is associated with the almost complete removal of the water content of the slices. The chips were kept submerged in oil by the use of a wire whip. When done, they were removed by means of the same utensil and allowed to drain on paper towels until records of color were taken.

When one sample of 10 chips was cooked it was immediately followed by the set of duplicates. The similarity of the members of each pair representing one tuber proved that the whole cooking process was sufficiently controlled to give uniform and comparable results.

COLOR SCALE

When the first controls, lot 1, were fried, five chips were selected to cover the range of colors represented. These were numbered in order beginning with the lightest. When lot 2, which had been held in cold storage two weeks, was fried the color range was extended in the direction of darkness, and two more chips were added to the scale.

⁴ Tests showed that this variation in time of soaking did not alter the frying color.

Finally an eighth very dark chip was added when lot 4, which had been in cold storage four weeks, was sampled.

It should be emphasized that the purpose was merely to get some standard for comparing color differences. Because of the method of selection of these chips, it was impossible to be certain that they represented uniformly spaced degrees of change.

To standardize this scale the method of color measurement described by Nickerson (5) was employed. This gave color qualities according to the Munsell notation.⁵

TABLE 1. *Color qualities of standard potato chips according to Munsell notation*^a

Chip No.	Hue	Brilliance	Chroma	Chip No.	Hue	Brilliance	Chroma
1	25 00	7 91	5 85	5	17 85	5 33	5 83
2	23 58	7 59	6 76	6	16 16	4 43	4 04
3	21 20	7 05	7 08	7	15 92	4 31	3 79
4	19 07	6 11	6 90	8	15 00	3 42	2 08

^a Hue: Redness, yellowness, etc., or the quality that differentiates a color from gray of the same brilliance.

Brilliance: Lightness or darkness of a color.

Chroma: Grayness of a color.

In this notation all hues are comprehended in a series of 100 units representing subdivisions of the 10 primary and secondary hues. Thus standard red may be designated as 5, standard yellow red as 15, standard yellow as 25, etc., and all possible hues may be represented by some whole number or decimal between 0 and 100.

The brilliance scale begins at 0, absolute black, and runs through middle gray to absolute white at 10. The notation for chroma begins at neutral gray, 0, and progresses by equal steps to the strongest possible saturation which varies with brilliance and hue.

Complete notation is written: Hue brilliance chroma.

In these readings the following standard Munsell disks were used: Yellow 8/9, yellow-red 6/8, neutral 9/, and neutral 1/.

The same color scale was used throughout the experiments. Each chip fried was compared with it and given the number that which it most nearly resembled. When the ratings for the 10 sets of duplicates were averaged, the result was used to represent the sample in the data that follow.

EXPERIMENTAL DATA

RELATION BETWEEN EXPERIMENTAL STORAGE CONDITIONS, CHIP COLOR, AND OTHER FACTORS

VARIETY AND CHIP COLOR

Six-ounce tubers from the Bliss Triumph, Irish Cobbler, and Green Mountain varieties grown on the two farms were compared in color of chips produced when they were held under the various storage conditions. The results are given in Table 2 as the average rating when compared with the color scale already described.

⁵ A "tuber line," as the term is used here, is a seed stock, perpetuated from year to year, that is known to have originated from a single tuber.

TABLE 2—Color of potato chips made from tubers of different varieties held under different storage conditions

Variety	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks, then—	
								One-half stored warm 4 weeks	One-half stored warm 8 weeks
Green Mountain (H) ^a ..	2 1 6	5 0	1 9	6 6	5 4	4 9	1 9	3 4	1 6
Green Mountain (A) ^b ..	4 1	6 7	4 5	8 0	6 3	5 6	4 0	4 1	3 4
Bliss Triumph (A) ..	3 6	6 8	4 4	8 0	7 0	6 8	4 0	6 0	5 0
Hush Cobbler (A) ..	2 9	6 1	2 9	7 0	6 2	6 0	3 0	4 8	3 0

^a (H) signifies that the potatoes were grown at Highmoor farm, (A) that they were grown at Aroostook farm.

^b For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

Chips made from potatoes from Aroostook farm were conspicuously darker than those from the tubers grown at Highmoor farm. Possible reasons for this will be discussed later. In all cases cold storage increased the darkness of the chips, while succeeding warm storage, if sufficiently prolonged, resulted in what was essentially a return to the original condition. Bliss Triumph tubers showed retardation in this reversion. The controls were very nearly constant throughout the experiment.

The probable errors of the means given in the tables were not computed because the numbering of the chips in the scale was a purely arbitrary method of characterizing differences not known to be uniform. In most cases variation within the samples was so limited that only two or three scale values were represented. The trends indicated by the means are unmistakable because their differences are so large.

SIZE OF TUBER AND CHIP COLOR

Four-ounce and 8-ounce tubers of the Green Mountain variety grown on both farms were selected for comparison with the 6-ounce sizes fried in the variety tests. These results are given in Table 3.

TABLE 3—Color of potato chips made from tubers (Green Mountain) of different sizes held under different storage conditions

Size (ounces)	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks, then—	
								One-half stored warm 4 weeks	One-half stored warm 8 weeks
4 (A) ..	^a 4 1	7 0	4 1	7 9	6 8	5 7	4 3	4 8	3 2
6 (A) ..	4 1	6 7	4 5	8 0	6 3	5 6	4 0	4 1	3 4
8 (A) ..	3 9	6 0	4 2	7 8	6 3	5 6	4 3	—	3 0
1 (H) ..	1 8	4 9	2 2	6 4	5 1	4 9	2 0	4 2	2 2
6 (H) ..	1 6	5 0	1 9	6 6	5 4	4 6	1 9	3 4	1 6
8 (H) ..	1 3	6 1	1 5	6 3	5 6	5 4	1 6	4 8	1 4

^a For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

There are no significant differences between the tubers of these sizes in color of chip after different conditions of storage.

TIME OF DIGGING AND CHIP COLOR

A series of Green Mountain potatoes grown at Highmoor farm was prepared to test the effects of varying maturity by digging at intervals during the season. The digging dates were August 6, 17, and 28 and September 16 and 26. The results are shown in Table 4.

TABLE 4.—*Color of potato chips made from tubers (Green Mountain) dug at different dates and held under different storage conditions*

Digging date	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 1 weeks, then—	
								One-half stored warm 4 weeks	One-half stored warm 8 weeks
Aug. 6.....	2 6	6 0	3 7	7 2	6 3	6 4	3 0	6 0	4 4
Aug. 17.....	3 4	5 5	3 3	7 0	6 5	5 9	3 2	6 0	4 2
Aug. 28.....	2 5	5 5	2 5	6 4	5 9	5 5	3 1	4 6	3 2
Sept. 16.....	1 8	5 8	1 9	7 6	6 3	5 4	2 1	1 8	3 2
Sept. 26.....	1 6	5 0	1 9	6 6	5 4	4 9	1 9	3 4	1 6

* For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

If potatoes are dug in an immature state they produce darker chips than do the more mature, even when they are held under similar storage conditions for a few weeks before frying. There is some evidence from these data that immature potatoes which have been in cold storage respond less slowly to warmth in returning to the original condition after a preliminary period of cold storage. Chips from immature potatoes are uneven in color, as are those which have been in cold, followed by warm storage.

GENETIC SIMILARITY AND CHIP COLOR

Two tuber-line ⁶ stocks were studied to see whether the tubers from them would exhibit uniformity in their response to changes in environment. The data for the individual tubers are shown in Table 5.

⁶ See footnote 5.

TABLE 5.—Storage effects on the color of potato chips made from tubers of two different genetic lines

LINE 1									
	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks then—	
								One half stored warm 4 weeks	One half stored warm 8 weeks
	3	7	2	8	6	6	1		
	3	6	2	7	7	6	1	3	3
	2	5	3	7	7	6	1	6	2
	3	6	3	8	6	5	3	6	2
	2	7	2	8	6	6	2	6	3
	2	7	3	8	6	5	2	6	2
	2	6	2	8	7	6	2		
	2	6	3	7	6	6	3		
	2	6	2	7	7	6	1		
	2	6	2	7	6	6	2		
Average	2.3	6.2	2.4	7.5	6.4	5.8	1.8	5.4	2.4

LINE 10									
	2	7	2	8	6	7	1		3
	1	7	2	7	6	6	2		3
	1	7	2	7	6	5	2		3
	1	7	1	7	6	6	1		3
	1	7	2	7	7	5	1		3
Average	1.2	7	1.8	7.2	6.2	5.8	1.4		3

* For meaning of numerals see discussion of color scale (p. 182). The larger the number, the darker the color.

Though the tubers of each line were genetically alike in so far as they were descended within three years from the same parent tuber, they did not respond in an identical manner to the conditions of storage.

HILL SELECTION AND CHIP COLOR

Some hills were dug as units and kept separate to compare the color of the chips made from tubers in such groups under the different storage conditions. The results are shown in Table 6.

TABLE 6.—Storage effects on color of chips made from tubers grown in a single hill

[Each lot is from one hill]

LOT 1, CONTROL						LOT 5, STORED WARM 1 WEEK AFTER COLD STORAGE FOR 4 WEEKS					
Tuber No	Weight	Length	Width	Depth	Color	Tuber No	Weight	Length	Width	Depth	Color
	Grams	Cm.	Cm	Cm			Grams	Cm	Cm	Cm.	
1.....	51	4.8	4.7	3.6	a 1	1.....	63	4.5	5.5	4.8	7
2.....	56	4.9	5.2	3.9	1	2.....	204	9.6	6.8	5.1	6
3.....	81	5.0	5.8	4.8	1	3.....	223	9.5	7.3	5.3	6
4.....	139	7.0	7.7	4.9	1	4.....	237	9.0	7.7	6.0	7
5.....	143	7.5	6.6	5.0	1	5.....	240	9.2	8.1	5.7	6
6.....	173	7.8	7.1	5.2	1	Average.....					6.4
Average.....					1						
LOT 2, STORED COLD 2 WEEKS						LOT 6, STORED WARM 2 WEEKS AFTER COLD STORAGE FOR 4 WEEKS					
Tuber No	Weight	Length	Width	Depth	Color	Tuber No	Weight	Length	Width	Depth	Color
	Grams	Cm.	Cm	Cm			Grams	Cm	Cm	Cm.	
1.....	23	3.6	3.7	3.1	7	1.....	129	6.8	6.8	4.5	6
2.....	41	4.6	4.4	3.4	7	2.....	198	6.0	7.5	7.5	6
3.....	80	5.0	6.1	4.3	5	3.....	298	10.5	8.1	6.3	6
4.....	81	4.4	6.0	4.4	7	Average.....					6
5.....	160	7.8	7.1	4.8	6						
6.....	170	7.4	7.4	5.2	6						
Average.....					6.3						
LOT 3, CONTROL						LOT 7, CONTROL					
Tuber No	Weight	Length	Width	Depth	Color	Tuber No	Weight	Length	Width	Depth	Color
	Grams	Cm.	Cm	Cm			Grams	Cm	Cm	Cm.	
1.....	24	3.7	4.0	2.7	4	1.....	11	2.2	3.0	2.3	3
2.....	59	5.2	5.1	3.6	2	2.....	17	2.7	3.2	2.7	3
3.....	68	5.8	5.4	3.9	4	3.....	87	5.9	5.5	4.2	3
4.....	78	5.2	5.9	4.2	2	4.....	110	6.4	6.5	4.7	1
5.....	123	7.2	6.2	5.0	3	5.....	118	6.5	6.3	4.8	1
6.....	212	8.9	7.4	5.4	2	6.....	120	6.6	6.6	4.6	2
7.....	209	8.7	7.5	5.5	3	7.....	149	7.2	7.1	4.9	1
Average.....					2.9	Average.....					2
LOT 4, STORED COLD 4 WEEKS						LOT 8, STORED WARM 4 WEEKS AFTER COLD STORAGE FOR 4 WEEKS					
Tuber No	Weight	Length	Width	Depth	Color	Tuber No	Weight	Length	Width	Depth	Color
	Grams	Cm.	Cm	Cm			Grams	Cm	Cm	Cm.	
1.....	32	4.5	3.8	3.3	8	1.....	34	3.9	4.3	3.3	4
2.....	81	5.7	5.6	4.5	7	2.....	39	4.7	4.1	3.2	3
3.....	145	7.0	6.8	5.0	7	3.....	62	4.8	5.5	3.8	3
4.....	147	6.6	6.9	5.1	7	4.....	50	4.8	5.4	3.4	5
Average.....					7.2	5.....	80	7.9	6.4	4.1	5
						6.....	90	6.1	5.7	4.6	3
						7.....	120	7.2	6.4	4.3	4
						Average.....					3.9

* For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

There is considerable variation in response to effects of storage on color of chips made from tubers produced in a single hill, in some cases more than in others. However, averages show the same trend indicated by the averages of the 10-tuber samples used in the other lots.

Records for the individual tubers were taken where the data are summarized as in Tables 2, 3, and 4, but, as these in Table 6 indicate, there is no correlation between size and shape and the color of the chips.

DISEASE AND CHIP COLOR

The foregoing data were secured on potatoes raised in plots in which there was no recognizable disease, or in which all diseased plants were rogued out early in the season, as explained in the discussion of selection of the potatoes. A less complete set of experiments was carried out on samples from hills where there was known disease and from healthy rows growing adjacent to such diseased plants. These data are given in Table 7.

TABLE 7.—*Effect of storage under different conditions on color of chips made from diseased potato stock, or healthy stock grown near diseased*

Growth conditions as related to disease	Control	Stored cold 2 weeks	Stored warm 2 weeks after cold storage 2 weeks
Spindle tuber (A) ^a	3 1	5 7	2 5
Spindle tuber (H)	1 4	3 3	2 2
Healthy next spindle tuber (A)	3 5	6 9	3 4
Healthy next spindle tuber (H)	2 2	3 5	3 4
Leaf roll (A)	3 1	6 0	3 8
Leaf roll (H)	2 8	4 1	2 2
Healthy next leaf roll (A)	4 6	7 0	4 0
Healthy next leaf roll (H)	2 3	4 9	2 9
Rugose mosaic (A)	4 0	5 5	5 0
Rugose mosaic (H)	2 3	2 1	2 6
Healthy next rugose mosaic (A)	4 3	6 3	3 5
Healthy next rugose mosaic (H)	1 8	3 0	3 1
Mild mosaic (A)	3 3	5 0	3 5
Mild mosaic (H)	1 2	3 4	2 8
Healthy next mild mosaic (A)	4 7	6 4	3 4
Healthy next mild mosaic (H)	1 9	3 4	2 4
Giant hill (H)	2 6	5 7	4 3
Healthy next Giant hill (H)	2 2	5 4	2 6
Healthy (A)	4 0	6 4	3 9

^a (A) = potatoes grown at Aroostook farm, (H) = potatoes grown at Highmoor farm.

^b For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

In a few cases disease apparently affected the rate of change of frying color. Both samples of rugose-mosaic tubers failed to exhibit much change in the period allowed. One sample from healthy stock planted near stock having this disease showed this effect. These results should be tested further before they are considered especially significant.

CORRELATION OF CHIP COLOR WITH SUGAR CONTENT OF TUBERS

Five tuber lots of Green Mountain, from which sample chips corresponded to each color in the scale, were analyzed for their sugar content. The results are given in Table 8.

TABLE 8.—*Sugar content of Green Mountain tubers producing chips of standard colors*

Chip No ^a	Total sugar in tubers ^b (wet weight)	Chip No ^a	Total sugar in tubers ^b (wet weight)
	Per cent		Per cent
1	0.19	5	0.60
2	.27	6	2.87
3	.36	7	3.14
4	.48	8	3.25

^a See discussion of color scale for significance of numerals (p. 482). The larger the number, the darker the color.

^b Sugar analyses made by E. R. Tobey, research chemist, Maine Agricultural Experiment Station.

The higher the sugar content of the potatoes, the browner were the chips made from them. Thus low sugar content appears to be associated with lightness of color, whereas potatoes containing a high sugar content produce dark chips.

The comparatively large increase in sugar content of tubers corresponding in chip color to No. 6 as compared with No. 5 is not surprising when one recalls that chip No. 6 was selected from those made from tubers that had been in cold storage, while the first five chips were made from tubers as they came in from the field.

The correlation of browning during cooking at high temperatures with the sugar content of potatoes is similar to the condition found in baking bread. The amount of residual sugar escaping yeast fermentation depends upon the diastatic activity of the flour. Flours possessing high diastatic activity have high residual sugar and consequently make breads which brown easily during baking (3).

TRADE PREFERENCES IN CHIP COLOR

In order to ascertain trade preferences in color of potato chips, nine sets of chips similar to those used in the color scale for rating the experimental products were prepared and sent out to a corresponding number of manufacturers. The eight reports that were returned are summarized in Table 9.

TABLE 9.--Trade preferences for potato chips according to color

Manufacturer No.	Colors rated -			Manufacturer No.	Colors rated		
	Most desirable ^a	Market- able	Unmarket- able		Most desirable ^a	Market- able	Unmarket- able
1.	1	1, 2, 3	4, 5, 6, 7, 8	5.			
2.	1, 2, 3	1, 2, 3, 4	5, 6, 7, 8	6.			
3.	1, 2	1, 2, 3	4, 5, 6, 7, 8	7.			
4.	2, 3	1, 2, 3, 4	5, 6, 7, 8	8.			

^a See discussion of color scale for significance of numerals (p. 482). The larger the number, the darker the color.

Potato-chip manufacturers agreed that the trade prefers a light chip, so light that in a northern climate potatoes may be undesirable for this purpose even at digging time. (Compare these data with those in Table 2.)

From Table 8 it also seems that in the potatoes sampled, a sugar content of one-half of 1 per cent or over is associated with a chip that is unmarketable on account of its color.

DISCUSSION

It is well known that potatoes freeze at about 29° F. (11), and that the formation of ice crystals causes definite injury to cooking quality, but it is not so widely appreciated that storage at temperatures well above this will cause internal chemical changes which affect culinary value. In his study of the carbohydrate transformations in potatoes Appleman (1) found that sugar accumulates at the expense of starch when the temperature falls to 38°, and that the rate of accumulation is most rapid between 32° and 29°. In four to six weeks a maximum

of 3 to 4 per cent sugar is reached. Hopkins (4) found that at 40° the sugar content was fairly constant.

Stuart et al. (8) reported that the temperature of a potato-storage house on Aroostook farm varied between 34° and 45° F. during a winter, and so it is evident that storage houses in northern regions give conditions in which sugar accumulation takes place. Furthermore, such storage temperatures are considered desirable to prolong dormancy and to delay sprouting.

Applemen (1) also found that potatoes which have accumulated sugar at low temperatures will lose four-fifths of this by reconversion to starch and the remaining one-fifth through respiration in a room at 70° to 75° F. in a week's time. At lower temperatures, 45° to 50°, a much longer exposure is necessary. This phenomenon probably explains the lighter color secured when tubers from lots from which dark chips had been made were held at room temperatures. In this study the constancy of the controls indicates that the loss of sugar at the temperatures of the storage cellar (40° to 55°) was much smaller than the conclusions of Appleman (1) and Hopkins (4) would lead one to expect.

Appleman and Miller (2) in analyses of potatoes dug at different stages of development found that the percentage (wet weight) of reducing sugar plus sucrose was 0.991 in those dug on June 17, whereas those dug on August 27 contained only 0.255 per cent of these sugars. These were Irish Cobbler potatoes grown in Maryland, and the vines were brown and dry on the latter digging date.

The writer's experiments on making chips from potatoes dug at various times showed that the chips from those dug at the earlier dates were darker. (Table 4.) Again, this is a difference associated with relative sugar content. Differences in those dug earlier would probably have been more outstanding but for the period of holding before frying, as Appleman and Miller also say that the percentage of sucrose in their lots evened up by decrease during storage.

The factor of immaturity probably contributes to the difficulty in securing satisfactory chips from northern-grown potatoes even immediately after digging and before winter storage has produced its effects. Frequently these potatoes are dug while the vines are yet green. The difference in maturity may explain the difference in color of the chips made from Green Mountain potatoes produced at High-moor farm and those grown on Aroostook farm. (Tables 2 and 3.) The difference in soil temperature may also be a factor.

Since the caramelization of sugar is more rapid and complete at high temperatures than at low, the practicability of advocating lower frying temperatures was considered. Woodruff and Blunt (10) found that the overbrowning produced when potatoes were fried at 190° to 210° C. (374°–410° F.) diminished when they were fried at 170° to 190° (338°–374°). In the range of temperatures used, fat absorption did not vary with temperature.

In two tests on Green Mountain potatoes that had been in cold storage the author found that there was less browning when chips were fried at temperatures below 320° F. Fat absorption did not increase with lower temperatures until the temperature of the fat was below 250°. However, the sweetish flavor of the larger amount of sugar was evident and was objectionable.

SUMMARY

By employing the standard Munsell color disks it was found possible to measure the color of potato chips as a scale for comparison.

When potatoes of three varieties were held in commercial cold storage two weeks or more all produced chips very much darker than those made from tubers of the same lot cooked at the beginning of the experiment or held in storage at 40° to 55° F. If the potatoes which had been in cold storage were held at room temperature, the frying color became lighter. This change in color is correlated with change in sugar content under these different conditions of storage.

Potatoes dug at an early date so that they were somewhat immature gave chips darker than those from mature tubers. Size of tuber was not significantly related to chip color in the more mature lots.

Individual tubers from the same tuber line or from the same hill did not respond in an identical manner to those differences in storage temperatures. Averages were similar to those in other lots receiving comparable treatment.

Tubers from plants showing signs of the diseases spindle tuber, leaf roll, rugose mosaic, mild mosaic, and giant hill responded to cold storage like the healthy stocks already discussed except those having rugose mosaic. These did not show much change.

A survey of trade preferences showed that very light chips are desired. Northern-grown potatoes may be unsuitable for this purpose when they are brought in from the field. (See lot 1, Tables 2 and 3.)

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THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY IN 1929¹

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INTRODUCTION

The findings reported in this paper were obtained during the season of 1929 in the continuation of a project, the first results of which were reported about a year ago.³

For an outline of experimental methods connected with the project and detailed discussion of the results obtained in 1928 the reader is referred to the earlier paper.

Briefly, the conclusions reached were:

Application of fertilizer increased the quantity of dry matter produced and of all its constituents and improved the quality of the herbage.

Seasonal fluctuations in composition of the herbage were not smoothed out to any great degree by application of fertilizer.

Grass kept in the vegetative stage by grazing may vary considerably in composition as the growing season advances on account of the effect of such seasonal factors as rainfall, temperature, sunshine, and length of day.

TABLE 1.— *Kind of fertilizer used, rate, and date of application*

Plot No.	Kind of fertilizer	Pounds per acre applied	Dates of application (1929)
1, 2, 3, 5, 7, 8, and 9	Nitrophoska II " "	250	Apr. 20-May 2.
	Calurea " "	" 44	At intervals throughout the season varying from 26 days to 56 days, commencing May 28, ending Aug. 29.
	Calurea " "	44	
	Calurea " "	44	
4	Check	(^d)	
6	Acid phosphate	" 258	May 2.
	Muriate of potash	" 100	Do.

^a Nitrophoska II contains 16.5 per cent N, 16.5 per cent P₂O₅ and 20 per cent K₂O.

^b Calurea contains 34 per cent N and 13 per cent lime (CaO).

^c Plots 8 and 9 received a double application of Calurea (98 lbs. each) on Aug. 12 and 10, respectively, and none thereafter, thus making the total application to these two plots 132 pounds, the same quantity of Calurea as was applied to plots 1, 2, 3, 5, and 7.

^d No fertilizer.

^e These amounts of acid phosphate and muriate of potash supplied the same amounts of P₂O₅ and K₂O as did the Nitrophoska II.

¹ Received for publication May 9, 1930, issued October, 1930. Published with the permission of the director of the Massachusetts Agricultural Experiment Station.

² Grateful acknowledgment is here made of the cooperation of the Department of Animal Husbandry of Massachusetts Agricultural College and the feed control laboratory of this station, more particularly of the services of R. C. Foley, who took all samples, and of J. W. Kuzmeski and J. B. Zielinski, who made the determinations of crude fiber and ether extract under the direction of P. H. Smith, chief chemist of the feed control laboratory.

³ ARCHIBALD, J. G., and NELSON, P. R. THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY. Jour. Amer. Soc. Agron. 21. 686-699, illus. 1929.

EXPERIMENTAL PROCEDURE

The only important variations in the procedure in 1929 as compared with that in 1928, were the omission of nitrogen from the fertilizer application for Plot 6 and a 25 per cent reduction in the amounts of phosphoric acid and potash applied to the other fertilized plots. Also less nitrogen was applied in the spring and more during the summer. The detailed fertilizer schedule for 1929 appears in Table 1.

The system of grazing and of taking grass samples was identical with that in use in 1928.

DISCUSSION OF RESULTS

Results will be viewed from two angles: The effect of the fertilizer; and seasonal variations.

As in 1928, results from plots 7, 8, and 9 have been kept separate because these plots were not grazed during the early part of the season, being reserved for supplementary grazing acreage later in the summer. The grass on them was cut for hay in June. The cautionary note sounded in the earlier publication⁴ regarding interpretation of results is equally applicable here and need not be repeated. Table 2 shows the effect of the fertilizer treatment.

TABLE 2.—*Effect of fertilizers on the chemical composition (on a dry basis) of pasture grass grown on experimental plots in 1929*

[As in 1928 the values expressed in pounds per acre are averages of the weights of the crop at the several times of sampling. Obviously total weights would not be comparable because of the varying number of samples in the different groups]

Plot and fertilizer used	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre
<i>Plots 1, 2, 3, and 5</i>												
Average of 28 samples..... (Nitrophoska, Calurea.)	28.0	1,102	3.0	33.2	23.7	261	3.2	35.1	0.64	7.0	0.31	3.4
<i>Plots 7, 8, and 9</i>												
Average of 14 samples..... (Same fertilizer treatment as above, but not grazed until July.)	34.1	1,126	2.4	27.2	25.4	287	3.0	33.6	.67	7.6	.22	2.4
<i>Plot 6 (no nitrogen)</i>												
Average of 5 samples..... (Acid phosphate, muriate of potash.)	33.4	951	2.5	23.8	23.9	227	3.4	32.3	.65	6.2	.26	2.4
<i>Plot 4 (check)</i>												
Average of 5 samples..... (Unfertilized.)	39.8	971	1.9	18.4	26.7	260	2.9	27.9	.58	5.6	.27	2.6

Comparison of the figures shows that—

The grass from the check plot contained about 42 per cent more dry matter than did that from plots 1, 2, 3, and 5, but the average yield of dry matter on the dates when the samples were taken was about 13 per cent greater on the latter. Withholding nitrogen on plot 6 resulted in a dry matter percentage of 33 per cent as compared

⁴ ARCHIBALD, J. G., and NELSON. P., R. Op. cit., p. 689.

with 28 per cent in plots 1, 2, 3, and 5, and a dry matter production of 151 pounds less per acre, or, in other words, less succulent grass and less of it, even on a dry-matter basis. The considerably higher dry-matter content of the grass from all plots in 1929 as contrasted with 1928 can be attributed to a season as abnormally dry as that of 1928 was abnormally wet.

There was 58 per cent more nitrogen in the dry matter from plots 1, 2, 3, and 5 than in that from the check plot, and about 80 per cent more nitrogen was recovered per acre. This is an even greater response to the fertilizer than was obtained in 1928 and seems to point to one of two things or perhaps to both: (1) A residual nitrogen effect carrying over from the previous season, probably the result of a better developed sod on the fertilized plots, due to the fertilizer treatment in 1928; and (2) nitrogen applications may be more effective in a dry season than in a wet one.

That the first mentioned of these possibilities, viz, the residual nitrogen effect, must be at least in part responsible for the difference, seems evident from a comparison of the results from plot 6, which did not have nitrogen in 1929 but did have it in 1928, with the results from the completely fertilized plots and the check plot. A nitrogen content about midway between the two extremes was found in the grass from this plot, and an acre recovery of nitrogen about 29 per cent greater than the recovery on the check plot, albeit much below that on plots 1, 2, 3, and 5.

Production of crude fiber was about the same on plots 1, 2, 3, and 5 as on the check plot, but the percentage in the dry matter was somewhat less (23.7 as against 26.7), indicating that the grass had a higher nutritive value where the fertilizer was applied. Omission of nitrogen on plot 6 caused very little increase in fiber percentage—only 0.2 per cent. It should not be concluded, however, that nitrogen is unnecessary for production of high quality grass, for, as shown in the preceding paragraph, there was a very evident carry-over of nitrogen from the previous season on this plot.

While the crude fiber content of the dry matter of the grass from the fertilized plots (1, 2, 3, and 5) was about the same in 1929 as in 1928 (23.7 per cent and 23.5 per cent), there was a marked increase the second season for the check plot, 26.7 per cent as compared with 25 per cent. This is probably chargeable to the very dry season in 1929 and indicates that the check plot did not meet the severe test of the drouth so satisfactorily as did the fertilized plots. The very much higher dry-matter content on this plot in 1929 than in 1928 leads to the same conclusion.

The ether extract in the dry matter was about 10 per cent higher on plots 1, 2, 3, and 5 than on the check, and production per acre was about 26 per cent higher. Relatively these results are similar to those obtained in 1928, but despite the dry season the actual amount of ether extract produced was somewhat higher for all plots than in 1928. Regarding the intermediate position of plot 6 a deduction can be made similar to that already mentioned for nitrogen and crude fiber. Respecting the percentage content of ether extract, plot 6 was higher than the completely fertilized plots. It seems probable that this difference is relative only, because of the formation of less nitrogenous

substance where the nitrogen was withheld and a corresponding relative increase in nonnitrogenous substances.

The content of calcium was also about 10 per cent higher on the fertilized plots than on the check (a reversal of the findings in 1928), and recovery per acre was 25 per cent higher. The percentage and recovery of calcium were higher on all plots, the check included, than in 1928.

The phosphorus content was about 15 per cent higher on plots 1, 2, 3, and 5 than on the check, and recovery of phosphorus was 31 per cent greater. All plots showed a decrease in content but an increase in recovery of phosphorus as compared with 1928. The decrease in phosphorus content is possibly a reflection of the smaller amount of phosphate fertilizer applied in 1929 but is more likely due to the severe drought of July and August. Orr⁵ in a recent review of the subject cites several investigators whose work points to a lowered phosphorus content in pasture herbage during serious droughts.

With reference to plots 7, 8, and 9 the principal reason for the variation between these and plots 1, 2, 3, and 5 is a seasonal one, samples not having been taken from them until July. The relatively high calcium and low phosphorus which characterized these plots in 1928 persisted in 1929. As suggested in the earlier paper the high calcium may be due to the liberal application of calcium nitrate which these plots received in 1928. The low phosphorus may perhaps be accounted for by the fact that these plots have been brought under cultivation comparatively recently and as a consequence may not have accumulated a phosphorus reserve comparable with that on the first six plots, which had been fertilized quite liberally over a considerable period of years previous to the commencement of the grazing experiment.

To summarize briefly, the dry matter of the grass from the fertilized plots (1, 2, 3, and 5) was higher in total nitrogen, ether extract, calcium, and phosphorus, and lower in crude fiber than the dry matter of the grass from the unfertilized plot. Acre production of all constituents determined, crude fiber excepted, was markedly higher on the fertilized plots than on the check. For the most part the difference between fertilized plots and check was greater than in 1928. The effect of withholding nitrogen from plot 6 in 1929, as would be expected, was most apparent in the nitrogen results from that plot.

SEASONAL VARIATIONS

A summary of the seasonal variations appears in Table 3. The rainfall and temperature records at Amherst for the growing season of 1929 are shown in Figure 1 and have been given careful consideration in accounting for variations in composition. Variations in the several constituents are considered in detail below and are further illustrated in Figures 2-7.

⁵ ORR, J. B. MINERALS IN PASTURES AND THEIR RELATION TO ANIMAL NUTRITION. p. 41-44. London. 1929.

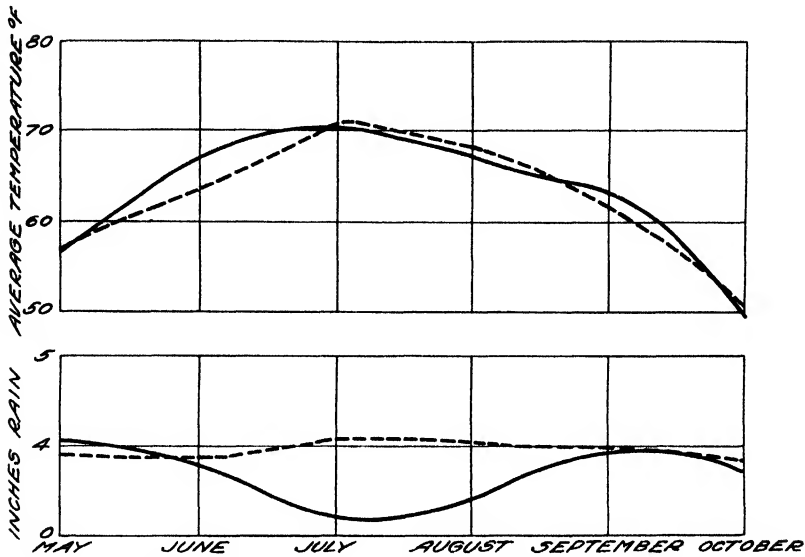


FIGURE 1 — Rainfall and temperature records at Amherst during the growing season of 1929. Dotted lines represent normals for Amherst, these vary slightly from those given for last year since a new 10-year normal has been established, whereas the 1928 weather graphs were based upon the 25-year normal formerly used by this station

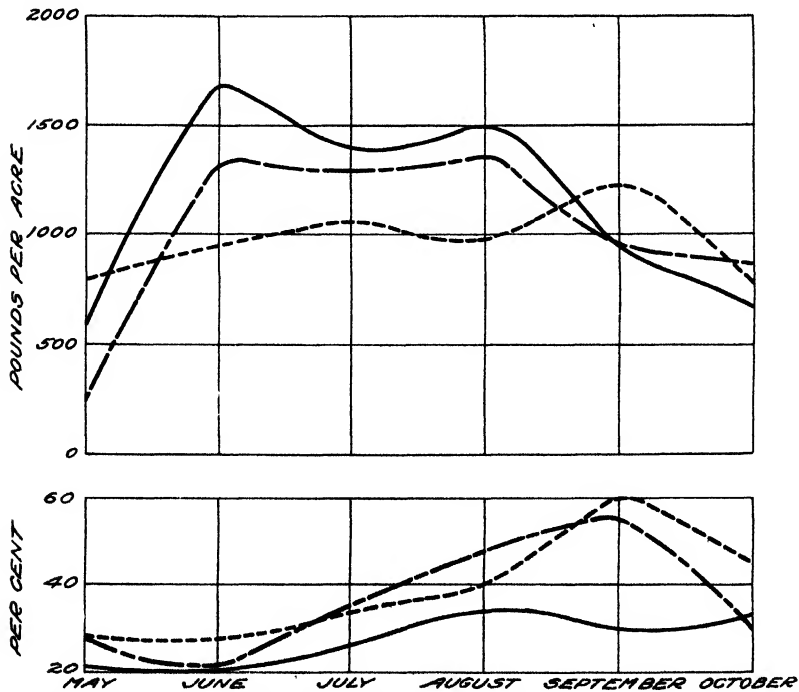


FIGURE 2.— Variations in average percentage content and production in pounds per acre of dry matter in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

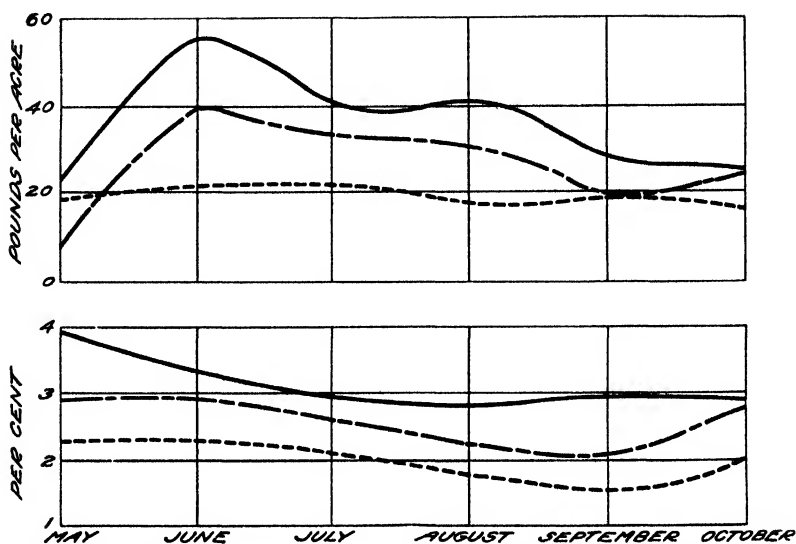


FIGURE 3.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of nitrogen in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

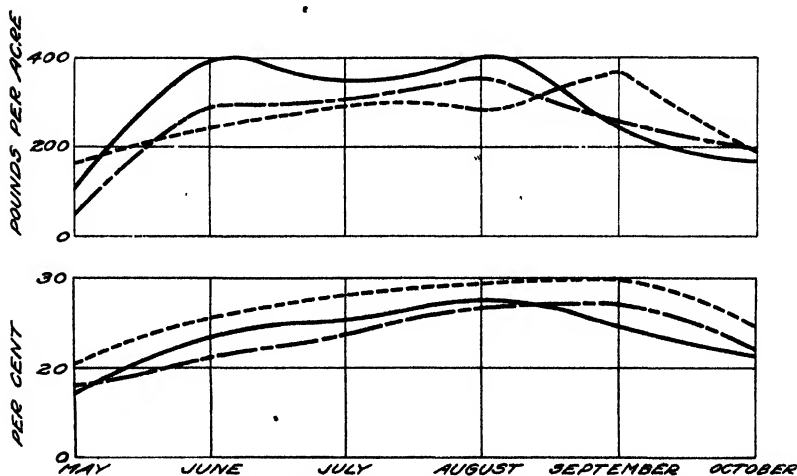


FIGURE 4.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of crude fiber in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

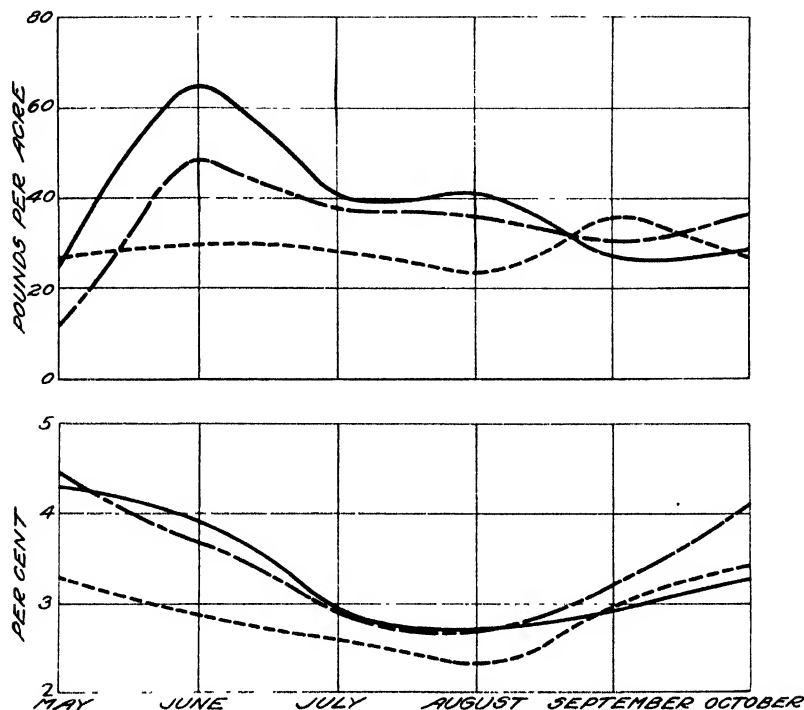


FIGURE 5.—Variations in average percentage content and recovery in pounds per acre, on a 'dry' basis, of ether extract in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot.

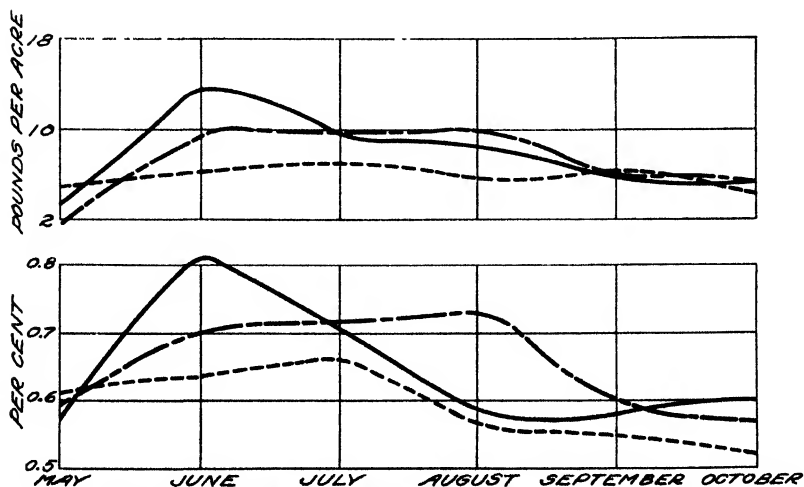


FIGURE 6.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of calcium in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot.

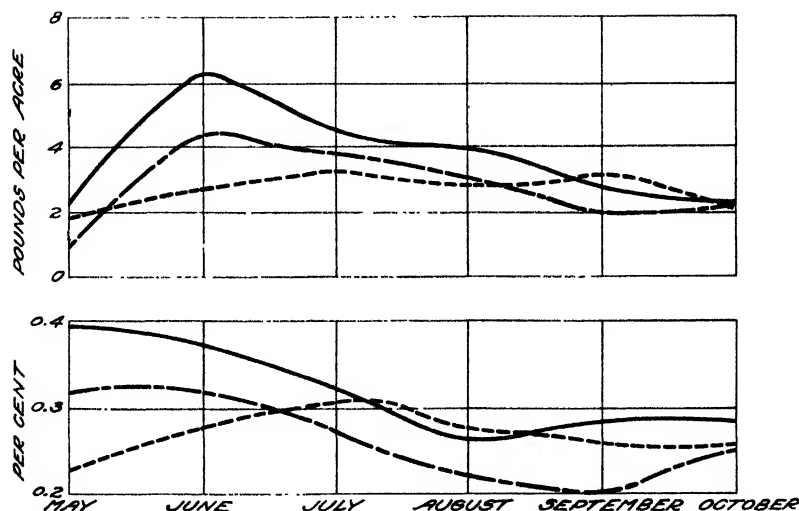


FIGURE 7.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of phosphorus in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot.

TABLE 3.—Seasonal variations in chemical composition (on a dry basis) of pasture grass grown on plots fertilized with and without nitrogen and on check plots, in 1929

PLOTS 1, 2, 3, AND 5

Month	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre	Per cent	Lbs. per acre
May	22.0	583	3.9	12.7	17.4	102	4.3	24.8	0.57	3.3	0.39	2.3
June	21.3	1,682	3.3	55.0	23.1	388	3.9	64.7	.81	13.7	.37	0.2
July	27.4	1,385	2.9	39.9	25.1	348	2.9	39.9	.70	9.6	.32	4.4
August	34.6	1,491	2.8	41.1	27.3	407	2.7	40.8	.58	8.6	.26	3.0
September	29.6	933	2.9	27.3	23.9	223	2.9	26.6	.56	5.2	.24	2.6
October	33.6	676	2.9	21.3	20.4	170	3.3	27.7	.60	5.0	.24	2.3

PLOTS 7, 8, 9^a

July	28.5	1,315	2.4	31.2	23.0	343	2.9	37.6	0.86	11.3	0.23	3.0
August	41.9	1,219	2.3	24.3	27.6	337	2.8	33.7	.60	7.3	.21	2.5
September	32.7	1,002	2.4	21.3	21.8	218	3.2	32.4	.55	5.5	.21	2.1
October	41.6	1,023	2.4	21.5	22.8	213	3.2	32.8	.63	6.4	.21	2.1

PLOT 6 (NO NITROGEN)^b

May	27.8	298	2.9	7.8	18.1	48	4.4	11.7	0.59	1.6	0.32	0.9
June	22.0	1,323	2.9	38.6	21.7	297	3.7	48.4	.70	9.2	.32	4.3
July												
August	47.6	1,352	2.2	30.2	25.4	357	2.7	35.8	.73	9.8	.22	3.0
September	55.3	940	2.0	18.7	27.0	254	3.2	29.7	.59	5.5	.19	1.8
October	32.0	871	2.7	23.6	21.9	191	4.1	35.9	.57	5.0	.25	2.2

PLOT 4 (CHECK)^b

May	24.2	798	2.3	18.2	23.3	162	3.3	26.5	0.61	4.9	0.23	1.8
June												
July	34.1	1,062	2.1	21.9	27.8	295	2.6	27.9	.66	7.0	.31	3.2
August	39.7	991	1.8	17.3	24.9	246	2.3	22.9	.56	5.5	.28	2.8
September	60.1	1,223	1.5	18.8	29.8	366	2.9	35.7	.55	6.7	.26	3.1
October	45.6	776	2.0	15.7	24.3	189	3.4	26.6	.52	4.1	.26	2.0

^a As plots 7, 8, and 9 grew a crop of hay during the early part of the season, no samples were taken from them until July and the results from them are not included in the discussion of seasonal variations.

^b The monthly values for the check plot and the no-nitrogen plot can not be considered as reliable as those for the completely fertilized plots, for they represent analyses of only one sample, whereas the others are averages of from three to five samples.

^c No sample taken; plot not grazed that month because of insufficient growth of grass.

DRY MATTER

The most striking feature of the fluctuations in dry matter was the remarkable rise in percentage content as the season advanced, more especially in the check and no-nitrogen plots. This of course was a direct and immediate result of the very dry season. The effect of the September rains in lowering the percentage is also worthy of note. Along with the consistently lower dry-matter content of the grass on the fertilized plots should be noted the consistently higher production of dry matter on these plots in pounds per acre except at the end of the season. With the one exception of the check-plot value for June, production of dry matter was consistently higher on all plots than in 1928 and for the most part markedly so.

NITROGEN

Fluctuations in percentage content of nitrogen showed the same general trend as in 1928, although they were not so marked. The completely fertilized plots were consistently higher than the no-nitrogen plot, and it in turn was higher than the check plot respecting both nitrogen content and recovery of nitrogen all through the season. The effect of residual nitrogen on the no-nitrogen plot referred to on p. 493 is even more strikingly brought out by a study of the seasonal fluctuations. Of interest is the increase in nitrogen content in October on the check and no-nitrogen plots. This is attributed to the rains and warm weather of early September.

Fluctuations in nitrogen recovery on the fertilized plots were more marked than in 1928 although of a similar trend. The reverse was true of the check plot.

CRUDE FIBER

As the season advanced there was a quite uniform increase in the percentage content of crude fiber on all plots until August and on the check and no-nitrogen plots until September. In 1928 the peak was reached in July and August, respectively, or one month earlier. The difference is probably due to the marked difference in the two seasons.

This marked increase in fiber content as the season progresses was commented upon rather fully in the earlier publication and was the chief basis for a conclusion drawn at that time to the effect that "although grass may be kept short by grazing or other means, it does not follow that it will have a composition and nutritive value throughout the season similar to that it had at the start."

This viewpoint has been questioned by Ellenberger et al.⁶ on the ground that the grass may have been more mature later in the season. This possibility was recognized in our former publication where it was stated that "part of the variation may have been due to slight differences in stage of maturity of the samples." It must be reiterated here, however, that all samples were very immature when taken, being still in the early vegetative stage, and it is difficult to believe that a variation from 17 per cent to 27 per cent of crude fiber could be more than partly accounted for on the basis of stage of maturity. The conclusion drawn in the earlier paper that the process of fiber building in the plant "is, in part at least, seasonal and to that extent inde-

⁶ ELLENBERGER, H. B., NEWLANDER, J. A., and JONES, C. H. YIELD AND COMPOSITION OF PASTURE GRASS. *Vt. Agr. Expt. Sta. Bul.* 295, 68 p., illus. 1929.

pendent of the stage of maturity of the plant," seems to be further confirmed by the results in 1929.

Acre production of crude fiber showed very similar seasonal fluctuations in all plots irrespective of fertilizer treatment.

ETHER EXTRACT

On all plots the seasonal fluctuations in content of ether extract parallel very closely the amount of rainfall, showing how dependent is the synthesis of this particular constituent on the moisture supply. The same was true in general last year. The ether extract of plant materials always includes whatever pigments may be present, the most important one in grass being chlorophyll. When one reflects on how grass "greens up" after a rain, indicating rapid elaboration of chlorophyll, the relationship between content of ether extract and amount of rainfall is seen to be a rational one.

The fertilizer treatment maintained acre production of this constituent at a much higher level during the first part of the season, but seasonal variations were accentuated rather than diminished by it.

CALCIUM

The seasonal variations in calcium were entirely different from the results of 1928, again due presumably to great seasonal contrasts in moisture supply. On all plots a relatively low value was found in May, which on the fertilized plots suddenly increased to a much higher value in June, was slower in reaching a maximum on the check and no-nitrogen plots, and dropped rapidly from the maximum in all cases to values at the end of the season as low or lower than those found at the start. The same was substantially true for the acre recovery of this element.

PHOSPHORUS

Unlike calcium, phosphorus showed much the same seasonal trends as it showed in 1928. Fluctuations in the values for the fertilized plots showed an even more striking resemblance to those for nitrogen than they showed in 1928, and with respect to the check plot the same apparent inconsistency is evident as was commented upon in the earlier publication, viz, a low value at the beginning of the season, rising to a maximum in midsummer. This situation is hard to account for; last season any attempt at explanation was carefully avoided as it was considered unsafe to draw conclusions until further results had been secured. Since a similar result has been obtained the second year it may be safe to put forward a tentative explanation to the effect that the liberal supply of plant food on the fertilized plots had its maximum effect in the earlier part of the growing season, while the rate of metabolic activity was somewhat slower on the depleted check plot.

SUMMARY

Results of analyses of 52 samples of grass from nine 8-acre pasture plots that were fertilized and grazed intensively, are reported, discussed, and compared with results which were secured in the previous season (1928), and which have been published elsewhere. The fertilizer treatment was the same as in 1928 except that nitrogen was withheld from one plot that had a complete fertilizer that year, and

the amounts of phosphoric acid and potash were reduced 25 per cent on all plots. In other respects the experimental procedure was the same.

The fertilizer treatment decreased considerably the dry-matter content of the grass and the crude fiber content of the dry matter. All other constituents of the dry matter that were determined were increased, the nitrogen showing the most significant rise. Acre production or recovery of all constituents was increased, nitrogen being nearly double that recovered on the check plot. The effect of withholding nitrogen from one plot was very marked.

The conclusions reached in the earlier publication regarding (1) increase in nutritive value due to fertilization, (2) absence of any appreciable effect of the treatment in leveling seasonal fluctuations, and (3) changes in chemical composition of the grass as the season advanced, are substantiated and strengthened by the results of 1929.

A careful study of all results for both seasons, and correlation of these with the weather records leads to the further conclusion that of all the factors that influence the chemical composition of grass, producing as they do marked seasonal fluctuations, the amount of rainfall is the most important.

THE USE OF PRESERVATIVES TO PREVENT LOSS OF NITROGEN FROM COW EXCRETA DURING THE DAY OF COLLECTION¹

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INTRODUCTION

This study was undertaken at the request of Director E. B. Forbes of this institute, in the course of preparations for a series of mineral and nitrogen balances with cows, and had for its object the determination of a method of prevention of the loss of nitrogen from combined urine and feces. These excreta, as voided, were to be guided by a rubber apron and galvanized-iron hopper into an open galvanized-iron pan in the gutter behind the cow; and the conditions to be met by the treatment were that it be effective during an interval of 24 hours; and that the preservative be cheap and free from objectionable odor, and of such nature as to be easily handled, and as not to contain nitrogen or any of the several inorganic elements to be studied.

METHODS

In the preliminary tests samples of fresh feces and urine were mixed in equal proportions and allowed to stand in open vessels at about 70° F. for 18 hours. Kjeldahl determinations then revealed losses which ranged from 6 to 30 per cent of the total nitrogen present. This large variation in the loss of nitrogen from the several samples was caused by differences in surface exposed—the greater the surface in relation to the quantity of excreta the greater the loss of nitrogen. Nitrogen determinations in further experiments were easily performed with the aid of a Folin ammonia apparatus, as described later.

The sample of excreta was put into an aeration flask, and ammonia-free air was drawn over the surface of the sample and through standard sulphuric acid. After titrating the unused sulphuric acid with standard alkali the ammonia lost by the sample was computed. Fifty grams of feces and 50 c. c. of urine were found to make a convenient-sized sample.

The feces from milk-producing cows was sufficiently plastic to allow a sort of pipetting, by the use of a 12-inch piece of ½-inch glass tubing fitted internally with a movable section of rubber stopper. One end of the tube was closed with this rubber disk and thrust into a mass of feces. Then suction was applied at the open end of the tube sufficient to cause the feces to enter and to rise to a fixed mark. The tube was then introduced into the aerating flask, and the feces were expelled by pushing down the rubber disk with a glass rod. Thus the feces were deposited in the bottom of the flask without smearing them on the neck. To this portion of feces was added 50

¹ Received for publication May 23, 1930; issue 1 October, 1930.

c. c. of urine. A battery of flasks thus prepared was used for a series of preservative comparisons. Since 100 gm. samples were used, and the feces and urine mixtures contained about 1 per cent of nitrogen, the decigrams of nitrogen lost were expressed as per cent of total nitrogen lost—a procedure which is only approximately correct, but which was found useful for comparative purposes.

RESULTS

In Table 1 are given the results of several experiments with different preservatives as listed. The preservative was added in each case in the strength of a normal solution. Then the feces, urine, and preservative were well shaken together.

The temperature of the laboratory varied from 70° to 90° F. at the time of the several experiments.

At the lower concentrations copper sulphate, formaldehyde, and zinc sulphate were most efficient in preventing loss of nitrogen. At the higher concentrations all of the chemicals used showed some preservative action. Copper sulphate was very effective but was discarded because of its reaction with the zinc coating of the collection pans. The tests with formaldehyde and zinc sulphate showed that these preservatives also were effective, and that in sufficient concentration they reduced the nitrogen loss to negligible quantities.

TABLE 1.—*Influence of different preservatives in preventing loss of nitrogen from 100 gm. samples of feces and urine mixtures*

Preservative used	Loss of N, expressed as percentage of total N present, when the indicated quantity of preservative, expressed in cubic centimeters of normal solution per 100 gms. of feces and urine, was used				
	2	4	8	12	15
Copper sulphate	0.15				0.31
Boric acid	3.19				.08
Ferrous sulphate	3.19				.08
Formaldehyde		0.18	0.05	0.01	1.08
Phenol	3.19				.86
Salicylic acid	2.29				1.42
Toluene	2.43				.01
Zinc sulphate	.72	21	03	.01	

* Standard acid completely neutralized, and some ammonia lost.

Check tests without any preservative showed losses of 3.19+ per cent, 5.99 per cent, and 2.63 per cent, in association with the 2 c. c., the 12 c. c., and the 15 c. c. series, respectively.

The manner in which the feces and urine were mixed had a marked effect on the loss of nitrogen as shown in Table 2. The term "not mixed" as used in this table signifies that the urine and preservative were placed in the Kjeldahl flask and that the feces were added thereto without shaking. It is obvious that the thorough mixing of the feces and urine by shaking increases enormously the speed of the action by which the nitrogen is lost, unless prevented by the preservative. Also, in these experiments the loss of nitrogen from the feces and urine, placed together but not mixed, either with or without preservative, was very small.

TABLE 2.—*Loss of nitrogen from cow excreta, expressed as percentage of total nitrogen present, as affected by mixing the urine and feces, and by treatment with 2 c. c. of normal solution of different preservatives per 100 gm. of feces and urine*

Treatment of feces and urine mixtures	Copper sulphate	Boric acid	Ferrous sulphate	Formaldehyde	Phenol	Salicylic acid	Toluene	Zinc sulphate	No preservative (check)
Well mixed.....	0 15	3 19+	3 19+	0 84	3 19+	2 29	2 43	0 72	3 19+
Not mixed.....	.35	.72	.12	.10	.55	.46	.19		0 24

In Table 3 are given the results of several experiments in which large quantities of feces and urine were placed in the excreta collection pans. These results serve as a check on the previous smaller-scale experiments. Copper sulphate, zinc sulphate, and formaldehyde gave complete protection against loss of nitrogen when added to a homogeneous mixture of feces and urine. When these excreta were not stirred together, copper sulphate, the most efficient of the preservatives used, showed little, if any, effect in retarding the small loss of nitrogen which occurred. The copper plating of the galvanized-iron collection pans, however, suggested that the pans might not stand up under this treatment.

TABLE 3.—*Loss of nitrogen from large quantities of cow excreta, expressed as percentage of total nitrogen present, as affected by mixing the urine and feces, and by treatment with 2 c. c. of normal solution of different preservatives per 100 gm. of feces and urine*

Treatment of feces and urine mixtures	Copper sulphate	Zinc sulphate	Formaldehyde	No preservative (check)
Well mixed.....	None.	None.	None.	6 to 30
Not mixed.....	1 47			* 1 0 to 2 6

* Temperature variation from 70° to 90° F.

With the elimination of copper sulphate from further consideration, for practical reasons, it was decided to use formalin as a preventive of nitrogen loss. In Table 4 is given a summary of the experiments in which nitrogen was determined with the Folin apparatus and formaldehyde was used as a preservative. The amount of preservative used is given as cubic centimeters of normal solution, per cent as formaldehyde, and per cent as formalin, in the mixtures of excreta, together with the loss of nitrogen from the mixed excreta with and without the preservative.

TABLE 4.—*Results of experiments with formaldehyde as a preservative in preventing loss of nitrogen from feces and urine mixtures*

Quantity of formaldehyde used per 100 gm. of feces and urine mixture			Nitrogen loss expressed as percentage of total nitrogen present *	
Normal solution used	Formaldehyde	Formalin (37.5 per cent)	With preservative	Without preservative
C. c.	Per cent	Per cent	Per cent	Per cent
2	0.06	0.16	0.84	3.2+
4	.12	.32	.18	6.0
8	.24	.64	.05	6.0
12	.36	.96	.01	6.0
15	.45	1.20	.08	2.6

* Nitrogen determinations with Folin apparatus.

SUMMARY

Cow feces and urine, placed together, but unmixed, showed during an 18-hour period losses of from 1 to 2.6 per cent of the total nitrogen present. When these excreta were thoroughly mixed the losses varied from 6 to 30 per cent of the nitrogen.

Copper sulphate, zinc sulphate, and formaldehyde, when added in small concentrations to mixtures of feces and urine, were highly effective in preventing losses of nitrogen as ammonia.

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STUDIES ON INFECTIOUS HAIRY ROOT OF NURSERY APPLE TREES¹

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INTRODUCTION

The enlargements that commonly occur on underground parts of piece-root grafted nursery apple trees have received attention from numerous investigators.

Smith and his coworkers (25),³ in their extensive researches on crown gall, established *Bacterium tumefaciens* Smith and Town.⁴ as the organism whose several varying strains produce different types of crown gall on many plants, including apple. After giving careful consideration to the question of whether they should include in one rather variable species all the strains of crown-gall bacteria studied or should split them into different species, these workers (25, p. 157-158) concluded that—

* * * much further time will be required to decide positively whether it is best to regard all crown galls as due to variations of one polymorphous species, or whether they should be separated into two or more species * * * and for the present at least we prefer to leave the group undivided, merely indicating the various cultures for purposes of convenience by the name of the plant from which derived, as daisy, peach, poplar, etc.

After the work of Smith and his associates a widespread tendency developed to attribute to the crown-gall organism various apple overgrowths whose cause had not been clearly shown. Later investigations, however, initiated by Smith (24), have tended to modify this conception. Lek (9) has given a review of the literature on various overgrowths.

Studies on control reflect the complexity of the problem and suggest that several factors influence the development of overgrowths. Riker, Keitt, and Banfield (21) effected marked reduction in the number of malformed trees by wrapping well-fitted grafts with adhesive tape.

¹ Received for publication May 1, 1930; issued October, 1930. Published with the approval of the director of the Wisconsin Agricultural Experiment Station. These studies were begun as a part of the Wisconsin program in the crown-gall project supported cooperatively by the Crop Protection Institute, the Iowa State College of Agriculture and Mechanic Arts, and the University of Wisconsin, have been continued in cooperation with the U. S. Department of Agriculture. This work has been supported in part by a grant from the special research fund of the University of Wisconsin.

² Died May 3, 1929.

³ Reference is made by number (italic) to Literature Cited, p. 538.

⁴ Synonym: *Phytomonas tumefaciens* (Smith and Town.) Committee S. A. B. This name is used in the present paper.

Melhus, Muncie, and Fisk (13) reported increasing the percentage of smooth trees by the use of wedge grafts. Riker (18) suggested the influence of incompatibility between scion and stock for consideration in relation to some of the enlargements encountered. Wormald and Grubb (30) reported the reduction of knots following chemical and mechanical treatments. On the other hand, Waite and Siegler (29) reported increasing the percentage of smooth trees by the use of cloth and raffia wrappers with treatments in hydroxymercurichlorophenol. They attribute the reduction in overgrowths to the action of the chemical on the crown-gall bacteria.

Siegler (23) isolated cultures of bacteria from malformations on nursery apple trees and conducted inoculation tests with them. He states (23, p. 312): "Just how important a rôle this apple strain of *Bacterium tumefaciens*, if in fact it is a strain of that organism, plays in the formation of the woolly-knot type of malformation is still undetermined."

The present writers (20, 22, 35, 36) have made preliminary reports on the isolation of an organism which is different from *Phytomonas tumefaciens* and which causes hairy root. A more detailed report of their studies⁵ on hairy root and its causal organism is the purpose of the present paper.⁶

DESCRIPTION OF INFECTIOUS HAIRY ROOT

The infectious hairy root under consideration is most commonly observed on grafted nursery apple trees 1, 2, or 3 years old as they are dug for storage in the fall. The nurserymen have applied the names "hairy root" and "woolly knot" to these malformations because of the very large number of small roots protruding either directly from the stems or roots or from localized hard swellings that frequently occur at the unions. (Fig. 1.) In the very early stages of the development of these malformations the "hairy" roots are fleshy; in intermediate stages mixtures of the fleshy and fibrous types are found; and in the later stages the fibrous type predominates. Since it has been shown by Harris (4) that moisture is an important factor in determining whether a root will be fleshy or fibrous, additional work needs to be done to ascertain how much the hairy root organism influences the type of roots produced. The enlargement often found at the base of the roots in the later stages is usually covered by a definite cortex, which, however, may be obscure because of the profusion of small roots extending through it. The interior of the basal enlargement is hard, being composed largely of woody elements, which occur in an irregular cross-grain position and supply vascular connection with the roots. In many cases, especially between the roots, small pockets are found which inclose particles of soil and a large number of soil fungi and bacteria. Typical examples of hairy root that followed inoculations may be seen in Figure 2.

In addition to the hairy-root enlargements just described, there are similar malformations which resemble the knots from which the hairy roots emerge, except that the roots have not developed. Such knots have a typical structure characterized by a very deeply convoluted surface from which fissures may extend through a tortuous

⁵ The experimental work was done at Madison, Wis., unless otherwise stated.

⁶ Since this manuscript was prepared, additional papers dealing with this subject have appeared (2a, 2b, 14a, 17a, 23a).

path for some distance into the enlargement. These fissures often contain soil particles and a variety of soil microorganisms. Although

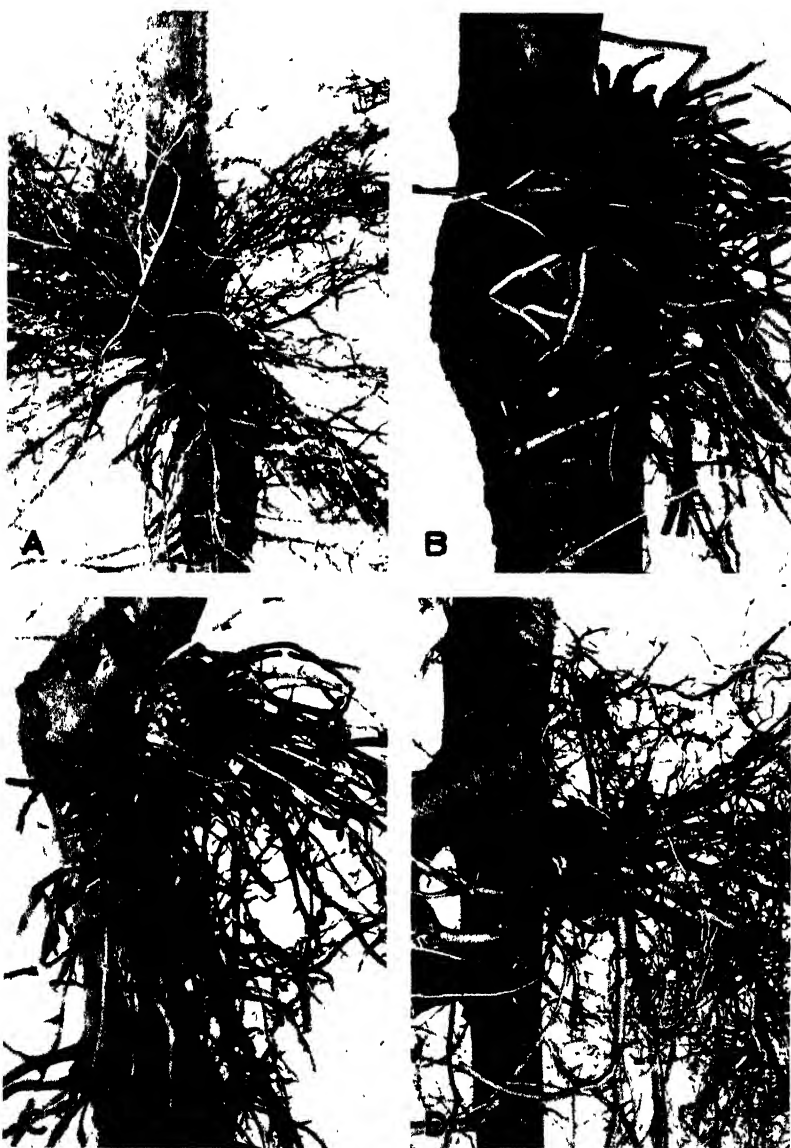


FIGURE 1.—Natural infection of hairy root on 3-year-old Wealthy apple trees from the nursery row. A and B.—Hairy-root development from little or no basal enlargement, showing predominance of fibrous roots in A and of fleshy roots in B. C and D.—Hairy-root development of the "woolly knot" type from comparatively small overgrowths

the etiology is still uncertain, typical hairy-root organisms have been obtained from this kind of knot and will be described later. A typical example is shown in Figure 3, D. Riker and Keitt (19) were uncertain

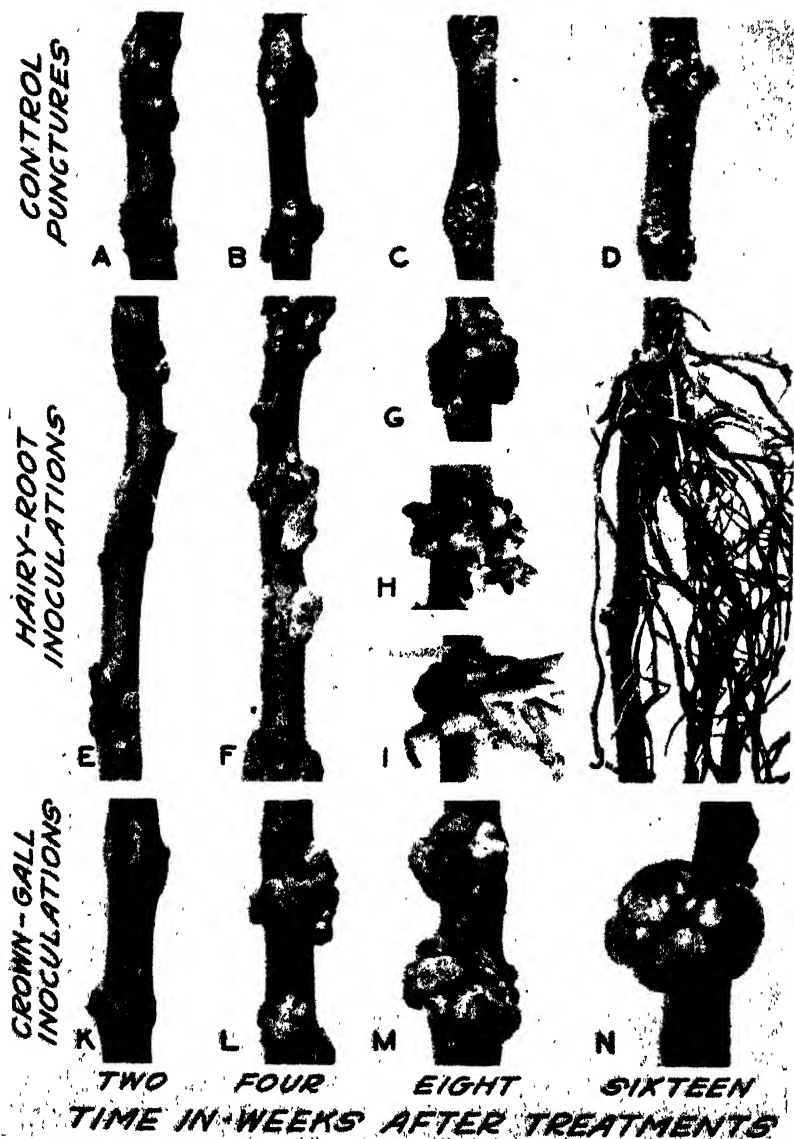


FIGURE 2.—Different stages in the development of enlargements on Wealthy apple scion wood below ground following certain treatments. A, B, C, and D.—Responses to uninoculated needle punctures at approximately 2, 4, 8, and 16 weeks after treatment. E, F, G-H-I, and J.—Responses to inoculations with the hairy-root organism at the same intervals of time. G, H, and I.—Different examples of the responses to this organism 8 weeks after inoculation. At the stage shown in G and earlier stages the response to the hairy-root organism might easily be confused with that to the crown-gall organism. At the stage shown in H the roots are beginning to appear, while in I they may have become somewhat elongated. K, L, M, and N.—Responses to inoculations with the crown-gall organism at the same intervals of time as the other series.

of the etiology of overgrowths of the two types just described, which yielded bacteria of doubtful identity. A continuation of the study

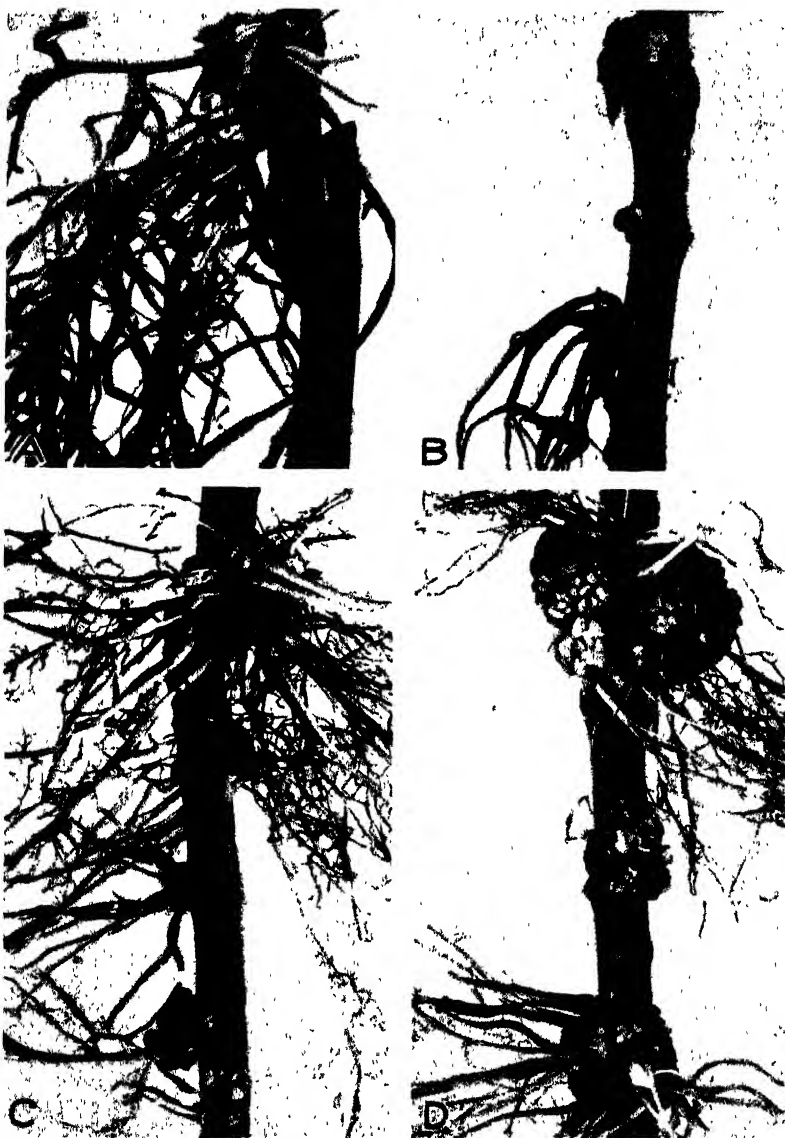


FIGURE 3.—Successive stages in the response of Wealthy apple grafts to puncture inoculations made with the hairy-root organism in the spring of 1927. A.—Dug in the fall of 1927. B.—Dug in the spring of 1928; shows immature roots which were winter killed and early stages of new hairy-root development. C.—Dug in the fall of 1928. D.—Dug at the same time as C; shows deeply convoluted type of overgrowth often found on nursery trees in their second year. Further work will be necessary to show conclusively whether or not this is an uncomplicated form of hairy root

of these cultures of unknown identity led to the investigation reported in the present paper.

Mixtures of the characters of the malformations just mentioned with those of overgrowths due to other causes are also found.

The very early stages of infectious hairy root on underground parts of nursery apple trees closely resemble those of crown gall and excess callus. Studies on the differentiation of crown-gall and hairy-root enlargements in these early stages of development on apple are not yet finished. In these stages it seems at present unwise to attempt such a distinction except as a result of bacteriological analysis. However, after a sufficient incubation period, the swelling induced by *Phytoplasma tumefaciens* becomes larger and shows no tendency to develop root primordia or roots. That induced by the hairy-root organism typically soon develops root primordia and roots. Illustrations of these types of malformation in different stages are shown in Figure 2. The characters of hairy root, crown gall, and wound tissue in their early stages are discussed further in the section on seasonal-development studies.

In inoculation experiments with the hairy-root organism on apple stems aboveground, malformations which resemble small burrknots have been induced. Siegler (23) reports similar results from inoculations with the cultures which he isolated from overgrowths on apples. In the present writers' experiments, although root primordia were sometimes produced, no roots were developed in the air except under artificial conditions of high humidity.

The resemblances to burrknots thus shown by malformations induced by inoculations with the hairy-root organism in the open air might raise a question (23) concerning the validity of the work of Brown (2), Riker and Keitt (19), and Hatton, Wormald, and Witt (5). The present writers, using Patel's method (16), have attempted isolations from 14 typical burrknots of various ages on apple received in 1928 from West Virginia through the courtesy of N. J. Giddings and his staff. In each case, in accordance with the work just cited (23), negative results were obtained. The majority of the isolation plates were completely sterile, and in no case was any colony secured which closely resembled the crown-gall or hairy-root organisms. The evidence now available seems to be against the conclusion that most of the common, naturally occurring burrknots are of parasitic origin. When burrknots occur beneath the surface of the ground and roots are produced from them, as for example on Paradise and Doucin stocks, the resulting formation is very similar to hairy root. As far as the present writers are aware a clear differentiation between these underground burrknots and hairy root on the basis of external characters has not yet been worked out.

Examinations of the hairy root of apple seedlings, which was studied by Muncie (14), have not been included in the work reported in the present paper.

ISOLATION AND INOCULATION STUDIES

Isolation and inoculation experiments with the hairy-root organism were begun in connection with the studies of crown gall and wound overgrowth of apple nursery stock reported by Riker and Keitt (19). By using a carefully standardized technic they found that *Phytoplasma tumefaciens* was readily isolated from a type of malformation which they considered to be the true bacterial crown gall and that the organism isolated induced typical crown gall when inoculated into

susceptible plants, such as tomato, geranium, tobacco, and apple. However, when the same technic was applied to apple malformations of various other types, very different results were obtained. In some cases the isolation plates either remained sterile or developed colonies of bacteria which induced no overgrowth when inoculated into tomato, geranium, tobacco, and apple. In other cases organisms were isolated which closely resembled *P. tumefaciens* in colony characters, but which induced only doubtful or slight overgrowth responses in inoculation tests. In all cases these inoculations were made on tomato, and in many instances on apple, tobacco, and geranium. In his criticism of this work because of the host plants employed, Siegler (23) apparently failed to notice (1) that, in addition to tomato, geranium, and tobacco, apple was also employed as a test plant for the organisms and (2) that organisms secured from malformations described as typical crown gall on apple were pathogenic on tomato, geranium, and tobacco, as well as on apple. Concerning the identity and possible relation to overgrowths on apple of those bacteria which resembled the crown-gall organism in colony characters but occasioned little or no overgrowth on tomato, conclusions were withheld, pending the results of the further studies which are reported in the present paper.

Further evidence concerning the nature of these bacteria was secured in 1927 by the present writers, using the cultures isolated by Riker and Keitt (19). Each of eight selected strains was inoculated by needle punctures into underground parts of the stems of first-season Wealthy apple trees. Six inoculations with each strain were made on each of 10 trees. Fifty trees were similarly punctured for controls but not inoculated. At the time of inoculation the experimental trees had made about 12 inches of new growth. In each case, after the treatments were completed, soil was heaped up over the places treated so as to provide as nearly natural conditions as possible for the incubation of the organism. After two months either hairy root or crown gall showed at the places of inoculation. When the trees were dug in the fall, six of the eight cultures had induced hairy root and two had induced crown gall at the places where the bacteria were inserted. The 50 control trees all gave negative results. The details of these studies are presented in Table 1. Attempts to recover each of the six strains of bacteria which stimulated hairy root from these artificially infected trees yielded bacteria which appeared on agar plates to be typical of those inserted. Cultures recovered from five of these overgrowths were reinoculated into apple in 1928. Each produced overgrowths typical of those from which the isolations were made.

These preliminary experiments made it seem desirable to undertake a detailed study of infectious hairy root. Trees bearing hairy root were secured from nurseries in different localities where it was prevalent and brought into the laboratory for examination.

The procedure in the inoculation work on infectious hairy root during the winter of 1927-28 was similar to that employed by Riker and Keitt (19). However, instead of nutrient dextrose agar or yeast-infusion agar, the medium devised by Patel (16) was used. This medium contains, per liter: Agar, 17 gm.; bile salt (sodium taurocholate), 3 gm.; glucose, 2 gm.; and crystal violet, 0.002 gm. It is adapted from the

well-known media employed in isolations of organisms of the colon-typhoid group.

TABLE 1.—Results of a series of puncture inoculations with cultures of the hairy-root and crown-gall organisms in 1927 ^a

Culture (organism and No.)	1-year-old wood			New wood		
	Number of inoculations	Result of inoculations		Number of inoculations	Result of inoculations	
		Hairy root	Crown gall		Hairy root	Crown gall
Hairy root						
1207.	30	12	0	20	15	0
2004	41	10	0	19	12	0
2014	34	5	0	25	21	0
2098	41	11	0	19	10	0
2104	47	12	0	14	7	0
2105	34	2	0	30	12	0
Crown gall						
2018	47	0	3	11	0	9
2124	47	0	3	11	0	9
Controls	128	0	0	112	0	0

^a On July 10 each culture was inoculated through needle punctures into both the 1-year-old scions and the new shoots of 10 first-year Wealthy apple trees in the field. Fifty control trees were punctured but not inoculated. All places of inoculation and all control punctures were promptly covered with soil. The results were taken Oct. 28.

The value of this bile medium was compared with that of yeast-infusion agar. The bile agar materially reduced the amount of growth of certain fungi and of some types of bacteria which often occur in the soil and within portions of the hairy-root overgrowths. It seemed to have no inhibitory effect upon the early development of *Phytoplasma tumefaciens*, the hairy-root organism, *Bacillus radiobacter* Beij. and Van Deld., or certain nonpathogenic mucoid organisms of the soil. However, these organisms were apt to die on the bile medium after some days. Consequently yeast-infusion agar was used to carry the stock cultures after they were transferred from the isolation plates. The efficiency of Patel's method (16), in which bile agar was used, was compared upon 20 different enlargements with that of the method employed by Riker and Keitt (19), in which yeast-infusion agar was used. The bile-agar method yielded positive results in 19 cases, while the yeast-infusion agar method gave positive results from all the specimens. This difference was considered to be within experimental error. Although Patel's method was found to be no more accurate in the hands of the present writers than the yeast-infusion agar method, it was used for routine work because of other advantages. Time was saved because only three isolation attempts, instead of five, were made for each enlargement, and difficulty with contaminating organisms was reduced.

During the winter of 1927-28 isolation studies were made upon 88 additional enlargements, chiefly of the hairy-root type and including "woolly knot," which were secured from 12 representative nurseries in Iowa, Kansas, Minnesota, Missouri, Nebraska, Oklahoma, and Wisconsin. Typical specimens are shown in Figure 1. After the isolation plates were poured and individual colonies had developed, certain typical colonies were selected and transfers made for stock cultures.

Each strain of bacteria taken from the isolation plates was later inoculated into apple and tomato plants. In some cases additional inoculations were made on tobacco. The apple was used because it is the host plant with which this investigation is most concerned and is susceptible to both crown gall and hairy root. The tomato was selected as a promising differential host for the crown-gall and hairy-root organisms. In order to minimize the possibility of natural infection, the apple trees were grown under the following conditions: (1) In the open field in ground where apples had not been grown before, (2) in field plots that had been steamed for 25 minutes and boarded up according to the method (7) commonly used for tobacco seed beds, (3) in virgin soil in pots in the greenhouse, and (4) in autoclaved soil in the greenhouse under conditions designed to exclude the chance intervention of any infectious organism. The data are reported without further reference to the conditions under which the apple plants were grown, as like results were obtained by each method used. The earlier inoculations on apple were made in stems of the preceding year's growth. After it was found that the use of stems of the current year's growth reduced the incubation period and greatly increased the chances of obtaining positive results, the inoculations were made in young shoots from first-year grafts. The tomato and tobacco plants were grown in pots in the greenhouse. The bacteria from agar slants were inoculated through needle punctures into the stems of the experimental plants, aboveground on tomato and tobacco and underground on apple. Five punctures were made in each inoculated and in each control plant. Two plants of the apple were inoculated with each bacterial strain tested, and one control plant was punctured but not inoculated. For inoculations into tomato and tobacco, one plant was used for each bacterial strain tested, and one control was left for every five plants inoculated. For purposes of comparison many parallel inoculations were made with carefully purified strains of *Phytophthora tumefaciens* that had been isolated from typical crown galls on apple and other plants.

The results of these isolation and inoculation studies are too voluminous for detailed publication. Records relating to each of the 88 malformations studied were kept under 92 headings. Inoculation tests were made with 359 strains of bacteria, an average of over 4 strains for each overgrowth. Two of the 88 enlargements yielded the crown-gall organism, as determined by inoculations on tomato and apple. Thirteen yielded no organisms that induced response following inoculation. One yielded an organism that occasioned host responses intermediate between those of hairy root and crown gall. The other 72 specimens all yielded organisms that induced reactions characteristic of the hairy-root organism following puncture inoculations on tomato (fig. 4, B) and apple stems (fig. 4, A). Control punctures were all negative. The reactions of the inoculated and control plants are discussed more fully in the section on seasonal development studies and are illustrated in Figure 2. The percentage of cultures from individual enlargements which gave positive results for hairy root varied with the different knots. Some of the overgrowths yielded organisms all of which were able to stimulate hairy root, some yielded only nonpathogenic cultures, and others yielded both kinds. Some of the results are summarized in Table 2, where the specimens

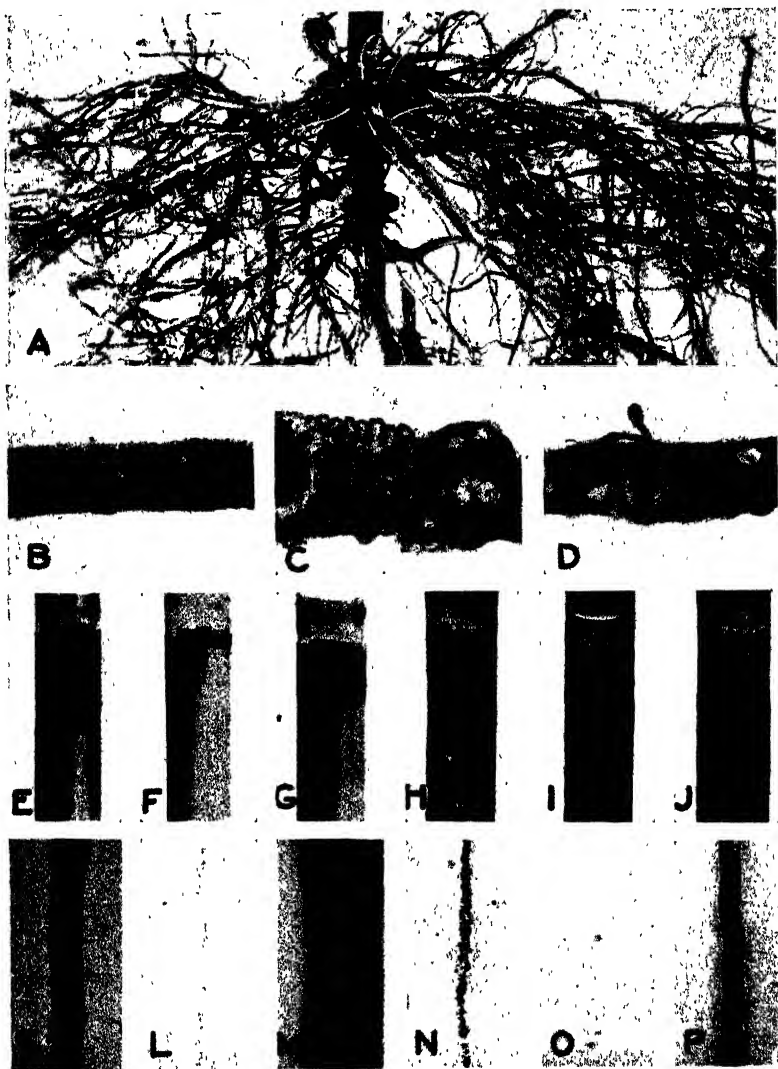


FIGURE 4.—Some results of inoculation and cultural studies of the hairy-root and crown-gall organisms and *Bacillus radiobacter*. A.—Wealthy apple graft four months after puncture inoculation with the hairy-root organism. B, C, and D.—Tomato stems one month after inoculation with the hairy-root organism, the crown-gall organism, and *B. radiobacter*, respectively. E, F, and G.—Growth characters of the crown-gall organism, the hairy-root organism, and *B. radiobacter*, respectively, in litmus milk after three weeks at 28° C. H, I, and J.—Growth characters of the crown-gall organism, the hairy-root organism, and *B. radiobacter*, respectively, in xylose peptone-salt liquid medium after four weeks at 21°. K, L, and M.—Growth characters of the crown-gall organism, the hairy-root organism, and *B. radiobacter*, respectively, in Petri dishes on glycerophosphate mannitol solid medium after two weeks at 21°. N, O, and P.—Growth characters of the crown-gall organism, the hairy-root organism, and *B. radiobacter*, respectively, in Petri dishes on glycerophosphate glucose solid medium containing brom-thymol blue, after two weeks at 21°. In N the bacterial growth was pale blue; in P, dark blue. There was little growth in O.

are classified according to the percentage of the strains of bacteria derived from them which gave positive results in inoculation tests.

In the work which has just been reported the hairy-root organism was isolated from a much higher percentage of the specimens cultured than in the earlier work reported by Riker and Keitt (19). In this connection the following facts are worthy of note: (1) As their investigation was not planned to include a study of hairy root, comparatively few specimens which showed excessive root development were included in the material which they studied, whereas in the present work on hairy root most of the specimens chosen for the isolation work showed excessive root development; (2) the use of improved methods of grafting, since the earlier work was done, has materially reduced the percentage of root-grafted trees which develop overgrowths, with corresponding changes in the percentages of these overgrowths in relation to cause; and (3) the inoculation technic used in the work reported in the present paper is more efficient in identifying the hairy-root organism than that used by Riker and Keitt.

TABLE 2.—Results of certain studies of the etiology of hairy root and "woolly knot" on apple, with the specimens classified according to percentage of strains of bacteria derived from them which gave positive results in inoculation tests, 1927-28*

Classes of specimens ^b	Specimens cultured	Average cultures per specimen	Cultures studied	Reactions of cultures on tomato and apple	
				Hairy root	Crown gall
A (100 per cent).....	43	3.8	162	157	5
B (50-100 per cent).....	18	5.0	90	59	2
C (15-50 per cent).....	14	4.1	57	14	0
D (0 per cent).....	8	5.6	45	0	0
E (0 per cent).....	5	1.0	5	0	0

* The sources of the material studied and the methods used in isolating the cultures and in making the inoculations are recorded in the text. These determinations are based upon the results taken from 1,148 inoculations on tomato and 3,104 inoculations on apple. The details of these studies are omitted because of their large volume. These cultures were secured by making transfers in the usual manner from well-isolated colonies in isolation plates, and received no further purification.

^b The classes are based on the percentage of strains isolated from each specimen which induced the reactions typical of either the crown-gall or the hairy-root organism when inoculated into tomato and apple. The numbers in parentheses show the range of the percentages included in each class.

The parallel inoculations with *Phytophthora tumefaciens* consistently yielded typical crown gall on apple, tomato, and tobacco. None of these plants developed hairy-root symptoms.

The etiology of hairy root has been further studied by means of reisolation and reinoculation experiments. Reisolation from hairy root induced by inoculation has been attempted from 40 specimens and has been successful in each case. The organisms secured from these isolations appeared typical of what was inserted. Thirty-eight representative cultures secured in this work from 12 experimentally produced hairy roots were reinoculated into tomato and apple. Cultures from each of these 12 specimens gave positive results following inoculations on apple.

Inoculation studies were also carried out with a series of crown-gall, hairy-root, and *Bacillus radiobacter* cultures that had been especially purified as reported later in this paper (p. 522). A number of different strains were inoculated by needle punctures into stems of tobacco and tomato. In the field they were inoculated just above the ground by needle punctures into the current season's growth of Wealthy apple grafts. These grafts had been planted in soil that had been steamed by the method described earlier. Immediately after

treatment the places of inoculation were covered to a depth of several inches with steamed soil. A summary of the results of these studies, together with the origin of the cultures used, is given in Table 3, which is discussed later (p. 534).

The limited data now available suggest that the hairy-root organism has a rather wide host range. Positive results have been obtained thus far from inoculations on the following plants, in addition to those already mentioned: Rose (*Rosa setigera* Michx.), honeysuckle (*Lonicera morrowi* Gray), sugar beet (*Beta vulgaris* L., var. *crassa* Alef.), bean (*Phaseolus vulgaris* L.), and Paris daisy (*Chrysanthemum frutescens* L.). Further studies of host range are in progress.

TABLE 3.—Results of puncture inoculations with certain replated cultures of the hairy-root and crown-gall organisms and *Bacillus radiobacter* on tomato, tobacco, and apple *

Culture		Tomato and tobacco				Apple	
Organism and source	No	Number of inoculations	Result of inoculations		Number of inoculations	Result of inoculations	
			Hairy root	Crown gall		Hairy root	Crown gall
Crown gall							
Rose	T-1	18	0	^b 5	20	0	0
Almond	T-2	27	4	^b 14	30	5	0
Incense cedar	T-3	27	0	23	50	0	0
Apple	T-4	18	0	18	15	0	0
Raspberry	T-5	45	0	45	40	0	7
Do	T-6	41	0	38	50	2	24
Willow	T-7	23	0	23	10	0	1
Peach	T-11	8	0	8	10	0	0
Raspberry	T-13	37	0	34	10	0	10
Apple	T-16				10	0	0
Peach	T-17	28	0	28	10	0	2
Raspberry	T-30	32	0	32	31	0	22
Do	T-33	15	0	^b 0	30	0	0
Apple	T-35	29	^d 0	29	45	^d 0	10
Peach	T-36	28	0	28	10	0	2
Walnut	T-37	28	0	28	65	2	10
Apple	T-39	18	0	18	10	0	0
Do	T-40	28	0	28	20	0	12
Hairy root, apple							
Do	T-31	8	0	0	20	0	0
Do	T-32	35	18	0	70	18	0
Do	T-34	24	6	0	70	1	0
Do	T-38	28	0	^d 28	70	33	^d 0
Do	T-41	19	9	0	55	43	0
Do	T-42	32	22	0	56	38	0
Do	T-43	21	13	0	65	42	0
Do	T-45	27	17	0	40	30	0
Do	T-46	28	13	0	45	36	0
Do	T-47	14	9	0	20	8	0
Do	T-48	23	4	0	20	13	0
Do	T-49	15	10	0	35	22	0
Do	T-50	23	18	0	40	30	0
B. radiobacter, soil							
Do	R-1	4	0	0	20	0	0
Do	R-3	4	0	0	30	0	0
Do	R-7	4	0	0	10	0	0
Do	R-9	4	0	0	10	0	0
Do	R-11	4	0	0	25	0	0
Do	R-16	4	0	0	20	0	0
Do	R-20	4	0	0	10	0	0
Do	R-27	4	0	0	10	0	0
Do	R-29	4	0	0	10	0	0
Do	R-31	4	0	0	10	0	0
Controls, punctures only		180	0	0	250	0	0

* Several subcultures were obtained from each original culture after the four successive replatings described in the text. The results from the subcultures are summarized for each original culture and reported opposite its number. T-5 was purified by the single-cell technique, the results shown being summarized for five strains.

^b It appears that the original cultures, which were positive for crown gall on tomato, were mixtures of the crown gall and some nonpathogenic organisms. In the process of purification the pathogenic organism was recovered in some instances, and the nonpathogenic bacteria in others.

^c In each of these cases one of the three (T-2) or four (T-6 and T-37) purified subcultures produced hairy root. It appears that the original cultures were mixtures of the hairy-root and crown-gall organisms.

^d Subcultures T-35-1A and T-38-1A appear still to be mixtures of the crown-gall and hairy-root organisms. The overgrowths that followed the inoculations were intermediate in characters between those typical of crown gall and hairy root. They are arbitrarily classified according to the characters which predominated.

SEASONAL DEVELOPMENT

Preliminary studies have been made on the seasonal development of hairy root following puncture inoculations, in comparison with the development of crown gall and the reaction to control punctures. During the spring of 1928, ground that had been used for truck crops in which crown gall is seldom if ever found in nature was selected for experimental plantings. This ground was prepared in the usual manner and then steamed according to the method in common use for tobacco beds (7). The steamed beds were then surrounded by 10-inch boards on edge to prevent the washing of surface soil. While these precautions did not exclude the possibility of the presence of the crown-gall and similar organisms in the experimental ground, it limited their access as much as might reasonably be expected under field conditions. In this steamed soil Wealthy apple grafts were planted as subjects for the study of the development of hairy root and crown gall following inoculation. Approximately 200 plants were inoculated with each organism by needle punctures through masses of the causal bacteria on the underground parts of the scion. When the current year's growth was tall enough inoculations were made also just above the ground on this new tissue. In most cases the needle was passed entirely through the stem. Soil was promptly heaped about the places of inoculation so that they were kept moist and under conditions favorable for root development. Four hundred control plants received the same treatment, except that no bacteria were applied. Attention such as spraying, cultivating, and irrigating was supplied as required throughout the growing season. The progress of the reaction to the different treatments was observed approximately twice a month.

Two weeks after treatment only a very slight difference was apparent between the reactions induced by the inoculations with the crown-gall and hairy-root organisms and those occasioned by the control punctures. In all cases slight enlargements had developed, which on the control plants appeared to be merely a callus response to the needle injury and which on the inoculated plants seemed at this stage to be little more than such a response.

A month after treatment the difference between the reactions of the inoculated plants and the controls was more marked. On the controls the enlargements were very little larger than at the end of the 2-week period, while those of both the hairy-root and crown-gall inoculations were almost twice as large as when last noted. The surface character of both of the latter at this time was of the crown-gall rather than the callus type. (Fig. 2, B, F, and L.)

Two months after inoculation a distinct differentiation in the responses to the three different treatments was evident. The control punctures had corked off in the usual manner, leaving a comparatively small development of wound tissue. (Fig. 2, C.) The crown-gall malformations had continued to enlarge, with the development of typical characters of crown gall. (Fig. 2, M.) No roots were observed to have developed from the gall proper. In exceptional cases in other experiments roots have been observed to push out from bud regions near the crown galls. Such root developments were also rarely observed near the control punctures. The hairy-root overgrowths at this time were in some cases merely enlargements of previous stages (fig. 2, G), while in others they showed the development of root

primordia and of roots (fig. 2, H and I). This root development occurred earlier upon the stems of the current year's growth than on older wood. It is important to note that up to and including this time the results of inoculations with the hairy-root organism on apple might readily be confused with those of crown gall.

Three months after inoculation the effects of the treatments were quite conspicuously differentiable. The control punctures showed no change. The crown galls were larger but showed no change in external characters. From the hairy-root inoculations, however, the roots had grown out in considerable numbers. Many of the early roots were fleshy. A mixture of fleshy and fibrous roots was quite common in the cases of hairy root studied.

Four months after the beginning of the experiment the overgrowths which resulted from the inoculations were observed to have enlarged without further changes in characters. (Fig. 2, D, J, and N.) Subsequent examinations showed additional growth of the hairy-root and crown-gall enlargements until very late in the fall. The white roots characteristic of new growth were apparent long after the top was frostbitten and at the time when the ground was beginning to freeze. These young roots were found to be very susceptible to winter injury. Harris (4) reports that young growing roots of apple are commonly very susceptible to injury from low temperatures.

The development of overgrowths following inoculations with the hairy-root organism over a 2-year period has been traced in ordinary field soil. (Table 4.) A considerable number of the trees used for the inoculations of 1927 (Table 1) were held over and grown a second year. In the fall of 1927 the places of inoculation showed abundant hairy-root development as previously described. (Fig. 3, A.) The winter of 1927-28 provided little or no covering of snow over the experimental planting at critical low-temperature periods, and the roots from hairy-root inoculations were killed. (Fig. 3, B.) However, they grew out again very early in the season of 1928, and by fall had matured into well-branched fibrous roots. (Fig. 3, C.) In addition to the second-year root development, peculiarly convoluted enlargements also appeared at many of the places of inoculation. (Fig. 3, C and D.) These were comparatively hard and contained deep fissures in which soil particles were frequently found to be incorporated. The convolutions often appeared superficially to be composed of a mass of rootlike tissue which seemed to grow together into a knot instead of growing out into roots. The histology of this structure has not been worked out. Macroscopically it is quite different from the typical soft gall which follows inoculations with *Phytophthora tumefaciens*. While these developments have appeared with some regularity at the places of inoculation in the second year, more evidence is necessary before they can be attributed without reservation to the hairy-root organism alone. They have appeared in a few cases subsequent to inoculations of the hairy-root organism into underground parts of slow-growing apple stems. From the overgrowths thus produced, bacteria were isolated which appeared to be typical of those inserted and which induced typical hairy root when reinoculated into underground parts of succulent apple stems.

TABLE 4.—A comparative record over a 2-year period of the development of hairy root and crown gall from inoculations on Wealthy apple nursery trees

Culture used	Trees treated	Inoculations ^a	Date observed	Overgrowths present			
				Hairy root	Crown gall	Doubtful	None
Hairy root, No. 2104.....	10	54	Oct. 28, 1927	19	0	14	21
			May 5, 1928	24	0	9	21
			Oct. 31, 1928	30	0	0	24
Crown gall, No. 2018.....	10	54	Oct. 28, 1927	0	9	7	38
			May 5, 1928	0	10	6	38
			Oct. 31, 1928	0	22	0	^b 23
Controls	5	30	Oct. 28, 1927	0	0	0	30
			May 5, 1928	0	0	0	30
			Oct. 31, 1928	0	0	0	^b 24

^a Hairy-root and crown-gall bacteria, respectively, were inoculated July 7, 1927, by groups of needle punctures into the underground stem parts of first-year apple grafts.

^b One tree died during the course of the second season of growth.

BACTERIOLOGICAL STUDIES¹

Since the crown-gall and hairy-root organisms showed such striking differences in the inoculation studies, it seemed desirable to subject them to a comparative bacteriological study. Accordingly, 18 of the cultures isolated in 1926 from apple and other hosts secured from a wide geographic range were run through the common bacteriological examinations. The results of this work showed that the cultures under consideration were somewhat variable in their characters and failed to show a clear differentiation between the crown-gall and hairy-root bacteria. This was entirely in accord with the conclusion reached by Smith et al. (25, p. 158).

Consideration of the data secured from the cultures used in 1926 suggested that either (1) the crown-gall species is highly variable or (2) the cultures employed were not pure. The colony characters of these cultures gave no suggestion of contamination. However, it was noticed that, on the media then employed, the colony characters of (1) the crown-gall organism, (2) the organism which was later found to cause hairy root, (3) *Bacillus radiobacter* (1, 11, 12), and (4) certain mucoid organisms common in the soil were indistinguishable. Furthermore, it has been commonly found by various workers, following Smith et al. (25), that the crown galls from the field contain various organisms in addition to the pathogene and that transfers from colonies typical of *Phytophthora tumefaciens* vary greatly in their pathogenicity (17). Each of the organisms just mentioned is of a slime-producing type exceedingly difficult to separate in pure culture by the ordinary agar-plate method. These and other considerations seemed to cast doubt upon the purity of the cultures used in 1926 and led to further efforts toward purification.

In questioning the purity of the cultures used in 1926 the writers do not wish to leave out of account the possibilities of variation in the progeny of pure cultures of bacteria. However, it seems unwise to ascribe variations in cultural characters to variability of the species, unless the best available methods for purifying the cultures have been

¹ The leadership in the bacteriological studies reported in this paper was carried by W. H. Wright, who died very suddenly when the preliminary draft of this work was almost completed. The other writers have finished minor phases of the experimental work and have completed the manuscript as nearly as possible according to Professor Wright's plan.

employed. It appears that the questions which the present writers have raised regarding the purity of their own earlier cultures may also apply to the cultures of other workers on crown gall who have relied upon the simple poured-plate method for purification. Because of the questionable purity of the cultures used in 1926, the results of the bacteriological studies made upon them are not given in detail.

The purification of the cultures at hand proved to be a difficult task. An attempt was made in 1927 to secure pure cultures by the isolation of single bacterial cells. The method of Wright and McCoy (32) was employed. Over 400 individual cells were isolated without serious difficulty, but less than 1 per cent of them grew. Consequently, this method was given up temporarily in favor of a poured-plate method in which special precautions were taken.

Each culture to be purified was transferred to a favorable liquid medium where it was hoped that the individual cells would grow separately rather than in aggregates held together in slime. Growth was found to be active in 18 to 20 hours after transfer, before any clouding of the medium appeared. Cultures for making dilution plates were used at this stage only. Some of the same kind of liquid medium in which the organisms were growing was placed in each of the series of Petri dishes to be used for different dilutions. The dilutions were made in the liquid medium in the Petri dishes so as to avoid the clumping effect sometimes produced by agar. The agar medium was then added and the plates were incubated at room temperature. Only plates which showed less than 50 colonies were used for making subcultures. Before the typical colonies were selected they were examined under the microscope for evidence of mixture of two colonies. Transfers were finally made to agar slants, where the growth characters were observed. This procedure was carried out three successive times with clear potato-glucose agar, and a fourth time with Kellerman's (8) nitrogen-poor Congo-red agar. The absorption of Congo red by all the colonies was not uniform after two weeks at 21° C. Notwithstanding this difference, reisolutions were made from the best dilution plates of all strains. Thirty-one strains of the crown-gall and hairy-root organisms and 10 of *Bacillus radiobacter* were purified in this way. Data concerning the origin and pathogenicity of the purified cultures are summarized in Table 3. Because of the difficulty of handling so many cultures for bacteriological studies, 18 of these cultures, which had been replated four successive times, were selected, as follows: Crown gall T-3-1c, T-5-1, T-5-3, T-6-1e, and T-35-1a; hairy root, T-32-2a, T-32-1b, T-34-1a, T-37-1b, T-38-1a, T-41-2b, T-42-1a, T-46-1b, T-48-1a, and T-49-1a; and *B. radiobacter*, R-3, R-11, and R-16. Cultures R-11 and R-16 were originally secured through the courtesy of N. R. Smith of the United States Department of Agriculture. T-5-1 and T-5-3 were the progeny of single-cell isolations.

The 18 cultures selected were compared in respect to morphology, colony characters, growth in various media, growth in litmus milk, growth on potato, limiting hydrogen-ion concentration for growth, absorption of dyes, rate of growth, nitrate reduction, fermentation of carbohydrates and related substances, and serological properties.

The cultures used for seeding the media in the various experiments were grown on clear potato-glucose agar slants for three or four days at room temperature. Equal quantities of suspensions, as uniform

as feasible, were always used in seeding the different series of culture media. Each strain of bacteria was run in duplicate in all of the experiments. All experiments, except the chemical analyses of the carbohydrate and alcohol fermentations, were repeated at least three times.

Long incubation periods were used in most of the experiments so that late changes would not be so likely to escape observation. This was done because preliminary experiments showed that the common periods of four to seven days were insufficient to bring out some of the important distinctive characters, especially those in litmus milk. All cultures were grown at room temperature unless otherwise noted.

The methods employed in making the different determinations, unless otherwise indicated, were those described in the Manual of Methods of the Society of American Bacteriologists (26).

MORPHOLOGY

The three organisms under consideration are typically small rods. Measurements made under standard conditions with a filar micrometer on organisms from 48-hour-old cultures on nutrient agar in smears stained with carbol fuchsin have given these figures: Crown-gall organism, $0.75\text{--}2.25\mu$ by $0.30\text{--}1.05\mu$, with an average of 1.43 by 0.60μ ; hairy-root organism, $0.55\text{--}2.59\mu$ by $0.15\text{--}0.75\mu$, with an average of 1.44 by 0.43μ ; *Bacillus radiobacter*, $0.30\text{--}2.25\mu$ by $0.15\text{--}0.75\mu$, with an average of 1.12 by 0.49μ . No particular significance is attached to the differences in the measurements of these organisms.

Examinations made of water mounts from 2-weeks-old cultures and by the use of Möller's method have both failed to reveal spores. In addition old cultures have been treated by heating for 10 minutes at 85°C ., after which transfers to both nutrient broth and potato-glucose agar slants showed no growth. Spores have never been found in any of the cultures.

Capsules have been demonstrated on the crown-gall and hairy-root bacteria and on *Bacillus radiobacter* with Gins's method (3). Abundant slime was found in cultures of these organisms and some difficulty was experienced before definite capsules were demonstrated.

With both Loeffler's and Casares-Gil's methods, each *Bacillus radiobacter* and each hairy-root organism was shown to bear a single polar flagellum. *Phytomonas tumefaciens* has yielded variable results with respect to the staining of flagella. Strains T-3-1c and T-6-1e, which were not of single-cell origin, had single polar flagella. However, repeated trials failed to demonstrate flagella on strains T-5-1 and T-5-3, which were progenies of single-cell isolations. None of the crown-gall organisms were found to be definitely motile in hanging-drop cultures. However, the hairy-root organisms were distinctly motile in hanging-drop cultures and *B. radiobacter* was actively motile.

Staining reactions showed all the cultures of crown gall, hairy root, and *Bacillus radiobacter* to be Gram-negative and not to be acid-fast.

COLONY CHARACTERS

All of the cultures were grown in meat-extract peptone broth, agar, and gelatin. Although they grew well in these media, there was very little difference in the appearance of the colonies or streaks of the three organisms. Clear potato agar containing 1 per cent of glucose gave excellent growth but did not differentiate them. The cultural

characters of colonies of each of the three organisms on this medium at 21° C. for seven days were as follows:

Surface type: Growth, rapid; form, circular (the size varied from 2 to 6 mm.); surface, smooth, shining; elevation, convex; edge, smooth, entire; internal structure, finely granular; optical characters, opaque to translucent, in some cases almost white.

Deep type: Form, mostly lenticular; internal structure, dense, nearly amorphous.

Bacillus radiobacter grow even more rapidly at first than either the crown-gall or the hairy-root strain. The colonies of *B. radiobacter* were often larger, nearly capitate, and more translucent. However, colonies of this organism were often convex, nearly white, and indistinguishable from colonies of the crown-gall organism.

GROWTH IN VARIOUS CULTURE MEDIA

In ordinary liquid media, such as plain and sugar broths, the growth characters of the three organisms were very similar. After 10 days or two weeks at 21° C. growth was shown by the formation of a heavy pellicle and a precipitate. This was more characteristic of the crown-gall and *Bacillus radiobacter* strains than of the hairy-root strains. The last named had a tendency to form less pellicle and sediment, with a more uniform clouding of the liquid. This difference was not well enough marked in these media to be of definite differential value.

Many synthetic media were tried with the anticipation that better cultural differentiation would be secured. One of the best for this work was a glycerophosphate medium devised by Lepierre (10) and used for a comparative study of several species of soil bacteria by Stapp and Ruschmann (27). This medium, with others, was altered in various ways to be used in both liquid and solid forms. The agar used in the solid media was washed in several changes of distilled water until the water-agar mixture showed no bacterial growth after standing in the 37° C. incubator for several days.

A peptone-salt medium was also found to be very satisfactory for growth of all the strains. Nine modifications of these two kinds of media, made according to the following formulas, were employed.

Medium I.—Glycerophosphate medium (Stapp and Ruschmann, 27): Mannitol 20.0 gm.; potassium nitrate (KNO_3), 5.0 gm.; sodium chloride (NaCl), 3.8 gm.; potassium chloride (KCl), 0.1 gm.; magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), 1.0 gm.; magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.6 gm.; calcium glycerophosphate, $\text{Ca}(\text{C}_2\text{H}_5(\text{OH})_2 \cdot \text{HPO}_4)_2$, 0.8 gm.; and distilled water, 1,000 c. c.

Medium II.—Same as Medium I, except that sodium glycerophosphate replaced calcium glycerophosphate.

Medium III.—Same as Medium I, except that potassium nitrate was omitted.

Medium IV.—Same as Medium II, except that potassium nitrate was omitted.

Medium V.—Same as Medium I, except that glucose was substituted for mannitol.

Medium VI.—Same as Medium I, except that no carbohydrate was used.

Medium VII.—Peptone-salt medium: Xylose, 5.0 gm.; magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.20 gm.; sodium chloride (NaCl), 0.2 gm.; calcium sulphate (CaSO_4), 0.1 gm.; dipotassium phosphate (K_2HPO_4), 0.2 gm.; peptone, 5.0 gm.; and distilled water, 1,000 c. c.

Medium VIII.—Same as Medium VII, except that glucose was substituted for xylose.

Medium IX.—Same as Medium VII, except that no carbohydrate was used.

All of the crown-gall, hairy-root, and *Bacillus radiobacter* strains were grown in the nine liquid media for 21 days at 21° C. Duplicate tubes containing 10 c. c. of medium were seeded with uniform suspen-

sions of the bacteria from cultures which had been grown on potato-glucose agar slants for two days at 21°. At the end of the incubation period each pair of duplicates was compared with a sterile control tube in respect to the following characters: Reaction (pH and total titratable acid), growth, pellicle, veil, and sediment. The summarized cultural characters of the three groups of cultures in four of these media are given in Table 5.

The most important differences between the three types of cultures (Table 5) are amount and type of growth in Media I, II, V, and VII. The 10 hairy-root strains were able to grow very little, if any, in the different glycerophosphate media. The crown-gall and *Bacillus radiobacter* strains made abundant growth in these media when potassium nitrate was present, but did not grow in Media III and IV, from which nitrogen was absent. They were likewise unable to grow when carbohydrates were omitted from Media V and VI. Neither the crown-gall nor *B. radiobacter* strains when grown in the glycerophosphate media showed significant change in pH values or titratable acidity.

TABLE 5.—Development of the crown-gall and hairy-root organisms and *Bacillus radiobacter* in glycerophosphate and peptone-salt liquid culture media, after 21 days at 21° C.

Culture groups	Cultural characters	Glycerophosphate media *				Peptone-salt medium *
		I Ca(C ₂ H ₃ O ₂) ₂ (OH) ₂ .H ₂ PO ₄ with mannitol	II NaC ₂ H ₃ O ₂ (OH) ₂ .H ₂ PO ₄ with mannitol	V Ca(C ₂ H ₃ O ₂) (OH) ₂ .H ₂ PO ₄ with glucose	VII With xylose	
Crown gall, 5 strains	Control, pH.....	7.0	7.0	6.4	6.4	
	Cultures, pH.....	7.4	7.4	7.2	6.3	
	Control, total acid ^b05	.05		.3	
	Cultures, total acid ^b05	.05		.35	
	Growth ^c	+++	+++	++	+++	
	Pellicle ^c	+++	+++	++	+++	
	Veil ^c	+	+	+	+++	
	Sediment ^c	++	+	+	+++	
	Control, pH.....	7.0	7.0	6.4	6.4	
	Cultures, pH.....	6.8	7.0	6.4	4.2	
Hairy root, 10 strains	Control, total acid ^b05	.05		.3	
	Cultures, total acid ^b05	.05		.62	
	Growth ^c	0	0	0	+	
	Pellicle ^c	0	0	0	+	
	Veil ^c	0	0	0	0	
	Sediment ^c	0	0	0	+	
	Control, pH.....	7.0	7.0	6.4	6.4	
	Cultures, pH.....	7.6	7.6	7.4	6.4	
	Control, total acid ^b05	.05		.3	
	Cultures, total acid ^b05	.05		.4	
<i>B. radiobacter</i> , 3 strains	Growth ^c	+++	+++	+++	+++	
	Pellicle ^c	+++	+++	++	+++	
	Veil ^c	+	+	+	+++	
	Sediment ^c	+++	++	+	+++	

* Roman numerals refer to media described under the same designations in the text.

^b The total acid is given in cubic centimeters of N/10 NaOH necessary to make 10 c. c. of medium neutral to phenolphthalein.

^c The amount of development is indicated as follows: 0, None, +, trace; ++, moderate; +++, abundant.

The growth of the three groups of organisms in the peptone-salt medium (VII) showed definite differences in amount and type. The abundant growth of the crown-gall organism and *Bacillus radiobacter*, with a pellicle, veil, and sediment, was in marked contrast to the

growth of the hairy-root strains, which was moderate and generally with little or no pellicle, veil, or sediment. (Fig. 4, H, I, and J.) No acid formation in the peptone-salt medium was caused by the crown-gall organism or *B. radiobacter*, even when glucose was present. In these cultures ammonia formation occurred and the reaction became slightly more alkaline. The hairy-root cultures, on the other hand, although showing less growth, and that of a different kind, caused a pronounced drop in the pH of the medium when glucose was present, even though some ammonia was produced. The responses of the three organisms in Medium VIII were similar to those in Medium VII. In Medium IX the hairy-root bacteria made slightly less growth than either the crown-gall organism or *B. radiobacter*.

The failure of the hairy-root strains to grow well on liquid glycerophosphate media was further tested with the same media containing agar. The liquid media were converted to solid by using 15 gm. of washed agar per liter. The agar was washed in many changes of water during two days at 37° C. The glycerophosphate agar was used with mannitol and with glucose. Streak cultures of each of the strains of the crown-gall and hairy-root organisms and *Bacillus radiobacter* were made upon the glycerophosphate agar containing mannitol and glucose. The cultures were grown as streaks in Petri dishes for 21 days at 21°. The differences shown by the three groups of organisms in the liquid media were even more conspicuous in the agar plate cultures. The crown-gall and *B. radiobacter* cultures grew well upon calcium glycerophosphate agar with mannitol, while the hairy-root strains were almost completely inhibited. (Fig. 4, K, L, and M.) The growth of the strains of *B. radiobacter* was usually heavier, raised, and more slimy than the growth of the crown-gall cultures. It was also usually surrounded by a halo of deep-brown pigment and an outer zone of a white precipitate. Streak cultures on calcium glycerophosphate agar, containing glucose and brom-thymol blue, brought out the same differences between the hairy-root strains and the others. (Fig. 4, N, O, and P.) The growth of the crown-gall and *B. radiobacter* cultures was not as abundant as with mannitol in the medium. The hairy-root strains were practically inhibited. The absorption of the dye by strains of *B. radiobacter* was greater than by those of crown gall. After about 10 days or 2 weeks a well-defined blue halo around the streaks clearly indicated an alkaline reaction.

GROWTH IN LITMUS MILK

Litmus-milk cultures of all of the strains were run in duplicate four times. In each instance the cultures were examined three times at intervals of one week. Variation in the growth characteristics of the crown-gall organism, when grown in litmus milk, has been reported by so many investigators that special care was taken to keep conditions constant.

Fresh skim milk containing 0.06 per cent of soluble litmus per liter was used for preparing the medium. Transfers were made with comparable suspensions of the bacterial growth from 48-hour-old agar plants, and the milk cultures were incubated at 28° C. The repeated trials with the three groups of organisms gave constant results.

The growth of all three groups of cultures was practically identical during the first week. The serum zone described by Löhnis and Han-

sen (12) as characteristic of *Bacillus radiobacter* cultures was produced also by the crown-gall and hairy-root organisms. Accompanied by pellicle formation it began to develop in all of the cultures in three or four days. After one week the serum zone was well-developed and varied from 3 to 6 mm. in depth. The color of the milk below the serum zone showed little alteration until near the end of the first week. At this time the litmus began to show a change of color and after two more weeks it appeared to be somewhat reduced. The crown-gall and the *B. radiobacter* cultures began to change toward an alkaline reaction while the hairy-root cultures were becoming acid.

After three weeks there was a well-developed difference between the crown-gall and the *Bacillus radiobacter* cultures on the one hand and the hairy-root cultures on the other. The serum zone continued to deepen in the case of the crown-gall and the *B. radiobacter* cultures, while it had a tendency to become shallower or to disappear altogether in the case of the hairy-root cultures. (Fig. 4, E, F, and G.)

A careful check of the cultures with brom-thymol blue, at the end of three weeks' growth, showed the crown-gall and the *Bacillus radiobacter* cultures to be decidedly alkaline and the hairy-root cultures quite acid. This decided difference in the growth of crown-gall and hairy-root organisms appears to be characteristic of them. Smith et al. (25) state in regard to the crown-gall organism, "Litmus milk is blued (never reddened) and the litmus is frequently reduced." In the present work this was found to be a consistent character of carefully purified crown-gall and *B. radiobacter* cultures. Pure cultures of the hairy-root organism, on the other hand, have always produced an acid reaction.

GROWTH ON POTATO

Growth of the three types of organisms on plain potato plugs, although not as characteristic as in litmus milk, showed that the hairy-root cultures grow differently from the crown-gall cultures. *Bacillus radiobacter* and the crown-gall organism showed a tendency to grow in a raised slimy mass along the needle track and between the sides of the tube and the potato piece. This was more characteristic of the *B. radiobacter* than of the crown-gall cultures. Browning of potato typical of *B. radiobacter* was observed also in the crown-gall cultures. Some of the *B. radiobacter* cultures seemed to cause more of this type of change than others.

Growth of the hairy-root strains on potato was watery, moderate to scant, and almost transparent. There was not the piling up or accumulation of a mass of growth such as was produced by the other types.

All these groups of organisms grew abundantly in the water under the potato plugs. The crown-gall organism and *Bacillus radiobacter* formed much more of a pellicle and precipitate in the water under the potato plugs than did the hairy-root organism.

LIMITING HYDROGEN-ION CONCENTRATION FOR GROWTH

The effect of hydrogen-ion concentration on growth was studied by varying the reaction of the following medium (31): Glucose, 5.0 gm.; magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.2 gm.; sodium chloride (NaCl), 0.2 gm.; calcium sulphate (CaSO_4), 0.1 gm.; dipotassium

phosphate (K_2HPO_4), 0.2 gm.; 10 per cent yeast infusion, 100 c. c.; distilled water, 900 c. c. This medium was prepared in quantities of several liters at a time and then divided into smaller quantities for each pH value to be used. Each lot of culture media was sterilized at pH 7.0 and then adjusted to the pH value desired by the addition of N/20 hydrochloric acid under aseptic conditions. The quantity of hydrochloric acid to be added to each lot of medium was first determined for a 100 c. c. sample of the same medium. After addition of the required quantity of acid each lot of culture solution was tubed under aseptic conditions. All of the various strains of the three groups of organisms were grown in duplicate in the culture solutions of each pH value. The technic of seeding these cultures was standardized by making water suspensions of the various strains, using organisms from 2-day-old cultures on potato-glucose agar slants. After thorough shaking 0.2 c. c. portions of these suspensions were used for seeding the cultures. At the time of transfer a careful potentiometric check of the pH value of each lot of culture media was made. The cultures were first grown in solutions decreasing from pH 7.0 to pH 4.0 in steps of 0.5 pH. In these solutions growth of all strains was abundant down to pH 5.0, and decreased somewhat but was still well marked at pH 4.5. A second series of cultures was prepared in the same manner as the first except that they were adjusted to the following reactions: pH 4.6, 4.4, 4.2, 4.0, 3.8, and 3.6. The final reaction was checked potentiometrically in each case, as before. Final results as to growth were recorded after two weeks at 21° C. The critical reaction (pH) for all three groups of organisms was between pH 4.4 and 4.0. The crown-gall and *Bacillus radiobacter* cultures showed a greater tendency to stop growth abruptly at pH 4.4, while five strains of the hairy-root organisms persisted in growing somewhat at pH 4.0. It was evident that there was not a great difference in the ability of the three groups of organisms to tolerate acid. The critical hydrogen-ion concentration was about pH 4.3.

ABSORPTION OF DYES

The absorption of Congo red by *Phytomonas tumefaciens* was observed by Kellerman (8) and later confirmed by Smith (24). In an earlier part of this paper reference was made to the use of Congo-red media in replating the cultures used in this work.

Yeast-infusion mannitol agar, to which was added an aqueous solution of Congo red to make a final concentration of 1 gram to 40,000 c. c., was used for the absorption tests. The various strains of the three groups of organisms were studied as ordinary colonies in loop dilution plates and as giant colonies in plate cultures. The dilution plates and giant colonies were made from suspensions of the organisms which had grown for three days on yeast-infusion mannitol-agar slants. The plates were incubated, under favorable moisture conditions, for 14 days at 21° C. The same differences were again observed as when the cultures were plated for purification. In general the three groups of organisms absorbed the dye in the following manner: Crown gall, strong; hairy root, moderate; and *Bacillus radiobacter*, none or a trace. As judged by intensity of coloring, the most absorption occurred in the crown-gall colonies from single-cell cultures. At first the colonies showed deep pink to red centers, but after two weeks, when the giant colonies had reached a maximum

size of approximately 20 mm., the coloring was uneven. In some cases concentric rings of color and in others radial sectors of deep red were plainly visible.

Absorption of Congo red by the smaller colonies in the loop dilution plates was not always distributed evenly throughout. Some showed intense red or pink centers while others were more uniformly colored. The subsurface colonies usually exhibited more pronounced shades, often an intense red.

Both giant and dilution-plate colonies of the hairy-root organisms showed much less tendency to absorb the dye. In some cases an accumulation of dye could be noticed in the central portions of the surface colonies, and deep colonies were often distinctly pink. The red color observed in the crown-gall colonies was not seen, although some of the pinkest of the hairy-root cultures were nearly as deep as the most lightly colored of the crown-gall cultures. In both cases the differences were most definite at the end of two weeks.

The three *Bacillus radiobacter* strains used for comparison absorbed very little dye during two weeks. The colonies remained translucent and slimy, the pink of the agar showing through them. Some of the deep colonies showed a more definite pink color, but none of them were distinctly red.

Brom-thymol blue, crystal violet, basic fuchsin, and methylene blue were not absorbed as selectively as was Congo red. With the exception of brom-thymol blue, the crown-gall cultures showed the strongest absorption of these dyes. When the culture medium contained glucose, and after the reaction became acid from fermentation of the carbohydrate in lightly buffered media, brom-thymol blue was quite strongly absorbed by the hairy-root strains. Variation of the reaction did not appear to affect the absorption of Congo red by the crown-gall organisms.

RATE OF GROWTH

Löhnis and Hansen (12) have reported that *Bacillus radiobacter* is a much faster growing organism than many of the nodule bacteria of leguminous plants. The similarity of the growth of the crown-gall and hairy-root organisms and *B. radiobacter* on favorable solid media suggested a comparison of the rate of colony growth of the three organisms.

The rate of growth of giant colonies of all the strains in each of the three groups was determined by measurement at regular intervals for two weeks. Yeast-infusion mannitol agar was used for growing the cultures, at 21° C. The final sizes and rates of growth of all three groups of organisms were very similar. The average diameters of these colonies were as follows: Crown gall, 18 mm.; hairy root, 20 mm.; and *Bacillus radiobacter*, 22 mm. The bacteria of crown gall and hairy root are of the rapid-growing type like *B. radiobacter*. The *B. radiobacter* colonies were often more raised (convex or capitate) than those of crown gall or hairy root, but in other respects very similar.

NITRATE REDUCTION

In the peptone-salt medium containing potassium nitrate and in the other media with nitrate, the crown-gall and hairy-root organisms did not reduce the nitrate. In the *Bacillus radiobacter* cultures, on the other hand, the nitrate completely disappeared. In this respect

B. radiobacter appears to be different from the crown-gall and hairy-root organisms.

In the peptone-salt medium and in other peptone-containing media, the organisms of all three groups produced ammonia. The ammonia was evidently formed from the peptone, for when peptone-free media were seeded with the organisms no ammonia formation took place.

FERMENTATION OF SUGARS AND RELATED SUBSTANCES

The amount of carbohydrates fermented by different organisms has been measured in various ways. The most common method has been to measure the acid and gas produced in the fermentation. None of the organisms studied has been found to produce gas. Further-

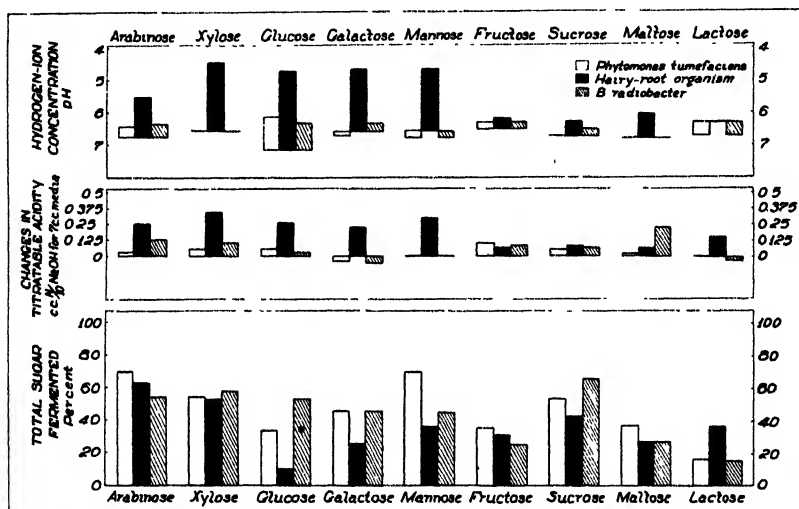


FIGURE 5.—Diagram showing the action of *Phytomonas tumefaciens*, the hairy-root organism, and *Bacillus radiobacter* on various carbohydrates as determined by hydrogen-ion concentration, changes in titratable acidity, and the percentage of total sugar fermented, as measured by quantitative sugar analyses. For each sugar fermented the hydrogen-ion concentration of the control is indicated by the continuous line through all three columns. The data on change in titratable acidity and on the percentage of sugar fermented are based, in each case, on the control as zero. The different sugars were subjected to the action of the bacteria in the peptone-salt liquid medium for three weeks at 21° C.

more, quantitative sugar determinations give a more satisfactory record of the amount of sugar fermented than can be had from records of gas formation and acidity. Consequently, in the present work it was decided to measure not only the changes in pH and titratable acidity, but also the quantitative changes in the amounts of reducing sugars present in the culture media.

The basal medium chosen for the studies on the fermentation of different carbohydrates was Medium VII, without xylose, which has been described. This medium was selected because it was favorable for the growth of all the strains of crown gall, hairy root, and *Bacillus radiobacter* employed. Five liters of this basal medium was made up to double strength. This was measured in 225 c. c. portions, placed in flasks, and autoclaved at a pressure of 15 pounds for one hour. The various carbohydrates employed were made up to 1 per cent strength in 225 c. c. portions of distilled water and autoclaved in the

same way. A flask containing 1 per cent carbohydrate solution was flamed and poured into a flask of the sterile double-strength basal medium. This mixture, containing the correct concentration of the basal medium and 0.5 per cent carbohydrate, was then transferred aseptically, in 10 c. c. portions, into sterilized test tubes. The media were incubated for a week to test sterility. The tubes containing 10 c. c. portions of the medium were seeded, respectively, in duplicate with the 18 strains of bacteria under study. Four tubes remained without seeding and were used as controls. The cultures were incubated at 21° C. for a period of 21 days before the carbohydrate fermentation was measured.

The fermentation of the carbohydrates was measured (1) by change in titratable acidity, (2) by change in pH, and (3) by quantitative determinations of the sugar in the cultures by the method described by Stiles, Peterson, and Fred (28). A list of the carbohydrates employed and a summary of the results of carbohydrate fermentation appear in Table 6. The action on reducing sugars is shown in Figure 5.

TABLE 6.—Action of the crown-gall and hairy-root organisms and *Bacillus radiobacter* on different carbohydrates, as shown by quantitative determinations of reducing sugars, by changes in hydrogen-ion concentration, and by changes in titratable acidity

Carbohydrate	Sugar fermented* by			pH concentration with				Change in titratable acidity † with—		
	Crown gall	Hairy root	B. radiobacter	Control	Crown gall	Hairy root	B. radiobacter	Crown gall	Hairy root	B. radiobacter
	Per cent	Per cent	Per cent					C. c.	C. c.	C. c.
Arabinose	66	60	52	6.6	6.3	5.1	6.2	+0.03	+0.23	+0.10
Xylose	52	50	55	6.4	6.3	4.1	6.4	+0.05	+0.32	+0.13
Rhamnose	---	---	---	6.6	6.6	5.6	6.2	-0.02	+0.08	-0.02
Glucose	32	9	51	7.0	6.0	4.7	6.2	+0.05	+0.25	+0.63
Galactose	43	24	43	6.4	6.5	4.6	6.2	-0.03	+0.17	-0.05
Mannose	66	34	43	6.1	6.6	4.6	6.6	.00	+0.28	---
Fructose	31	30	24	6.4	6.2	6.2	6.2	+0.10	+0.06	+0.07
Sucrose	51	40	62	6.6	6.6	6.2	6.4	+0.05	+0.07	+0.05
Maltose	35	25	25	6.6	6.6	5.9	6.4	+0.01	+0.05	+0.19
Lactose	15	34	14	6.2	6.6	5.1	6.6	.00	+0.13	-0.02
Raffinose	---	---	---	6.6	6.8	6.8	6.8	-0.07	-0.12	-0.05
Melzitose	---	---	---	6.2	6.2	6.4	6.4	-0.07	-0.12	-0.09
Starch	---	---	---	6.8	8.0	8.0	8.0	-0.15	-0.20	-0.20
Dextrin	---	---	---	6.2	7.2	7.3	7.1	.00	-0.03	-0.03
Inulin	---	---	---	6.1	7.2	8.0	8.0	-0.10	-0.17	-0.06
Salicin	---	---	---	6.2	6.1	4.6	6.2	.00	+0.17	.00
Aesculin	---	---	---	5.8	5.8	5.8	5.8	-0.10	.00	.00
Dulcitol	---	---	---	6.8	6.2	7.2	6.2	+0.03	-0.02	+0.03
Mannitol	---	---	---	6.4	6.4	6.3	6.5	.00	.00	.00
Erythritol	---	---	---	6.8	7.6	4.6	7.5	-0.07	+0.05	-0.10

* In each case control tubes were taken as 100 per cent.

† In each case the increase (+) or decrease (-) in acidity in comparison with the control tubes is recorded in c. c. N/10 sodium hydroxide. Each tube contained 10 c. c. of medium and phenolphthalein was used as the indicator.

‡ Repetition of this determination gave similar results. The low pH reading suggests that the growth of the organism was inhibited before much sugar was fermented.

The change in hydrogen-ion concentration is shown by giving the pH value of the control sample and the pH value of the culture after incubation. In the upper section of Figure 5 the continuous line across the three columns given for each sugar indicates the pH value of the control sample, while the variation from this line indicates the pH value after seeding and incubation. For example, the pH value

for the galactose control sample was 6.4, while the pH value of the medium after fermentation by the crown-gall organism was 6.5; by the hairy-root organism, 4.6; and by *Bacillus radiobacter*, 6.2. The change in titratable acidity was obtained by finding the difference between the quantity of N/10 NaOH necessary to neutralize to phenolphthalein the cultures and the controls after incubation for a period of 21 days.

An examination of the results from the fermentation studies shows several things. (1) Where sugar fermentation was determined quantitatively, crown gall and *Bacillus radiobacter* fermented approximately the same or a greater percentage of sugar than the hairy-root organism, except where lactose was used as a source of carbon. In this case the hairy-root organism fermented more of the sugar than either of the other two organisms. (2) Crown gall and *B. radiobacter* fermented the carbohydrates with but very little change in pH, while the hairy-root organism fermented arabinose, xylose, rhamnose, glucose, galactose, mannose, lactose, salicin, and erythritol with distinct decrease in pH. With these last-named carbohydrates the hairy-root organisms occasioned more change in titratable acidity than did either the crown-gall organisms or *B. radiobacter*. So far as the results of this study show, the crown-gall organism and *B. radiobacter* appear not to be differentiated from each other by means of their ability to ferment carbohydrates. Both produce fermentation with but little or no change in pH or titratable acidity. Whether these organisms are producing a neutral end product or enough ammonia from the peptone largely to neutralize any acid from the carbohydrates or are removing important ions from the medium remains to be determined. The hairy-root organism differs from the crown-gall organism and *B. radiobacter* in its ability to produce a decidedly acid condition in connection with the fermentation of certain carbohydrates.

Tests of the various strains with respect to hydrolysis of starch were made by growing streak cultures of the organisms on the basal medium for carbohydrate-fermentation studies plus soluble starch and agar. None of the cultures showed any diastatic action after 21 days at 21° C., when they were flooded with iodine solution. Eckford's method also gave negative results.

AGGLUTINATION TESTS

Agglutination tests were made of 5 strains of crown-gall bacteria, 2 of which were of single-cell origin; 11 strains of the hairy-root organisms; and 3 strains of *Bacillus radiobacter*. The rapid method described by Noble (15) and modified by Huddleson and Carlson (6) was used.

Cultures of the organisms for immunization of animals and for antigens were grown on neutral clear potato agar without glucose for four days. Rabbits were immunized with suspensions of these organisms in 0.85 per cent NaCl solution. The suspensions were made by washing the bacterial growth free from the agar, shaking, filtering through glass wool, and standardizing to a turbidity which would conceal a 2-mm. loop made from No. 26 B. & S. gauge wire at a depth of 12 mm.

Rabbits were injected intraperitoneally with the suspensions at intervals of four or five days until four injections had been used.

The amounts of injections were, respectively, 0.5, 1.0, 3.0, and 5.0 c. c. A week after the last injection each rabbit was bled from an ear vein. Since the test of the titer was sufficiently high in each case, the animal was bled from the ear until sufficient blood was secured for the agglutination tests. After coagulation of the blood in the ice box the serum was removed and preserved for the agglutination tests.

The heavy antigen was prepared in the same manner as the suspensions used for inoculation of the animals, except that the bacteria were suspended in 12 per cent sodium chloride, and phenol was added until the mixture contained 0.5 per cent. The heavy suspensions were made up to a turbidity which at a depth of 8 mm. concealed the 2-mm. loop made from No. 26 B. & S. gage wire.

Dilutions of antisera, 1:50, 1:100, 1:200, 1:500, 1:1,000, and 1:2,000, were used in preliminary trials. Agglutinations with homologous sera showed a titer of 1:2,000 in practically every case. The final agglutinations were run by making dilutions of 1:500, 1:1,000, and 1:2,000 with a calibrated capillary pipette. The results are summarized in Table 7.

One of the hairy-root cultures was omitted from the serological tests because it had failed to make satisfactory growth when the antigens were prepared.

Each of the 19 strains of the three groups of organisms employed was agglutinated by the antiserum from its own group but not that from any other group except in the case of two strains of the crown-gall bacteria, one of the hairy-root organism, and one of *Bacillus radiobacter*. None of these four aberrant strains was of single-cell origin. Of the two aberrant strains of the crown-gall organism one gave no agglutination with any of the sera, and the other cross agglutinated completely with the hairy-root organism and with neither the crown-gall organism nor *B. radiobacter*. In various tests this latter strain appeared to be a mixture of the crown-gall and hairy-root organisms, as explained in the footnote to Table 7. The one aberrant strain each of the hairy-root organism and *B. radiobacter* gave no agglutination with any of the antisera.

TABLE 7.—Results of agglutination tests with cultures of the crown-gall and hairy-root organisms and *Bacillus radiobacter*

Antigens		Results with sera from—			
Culture group	Number of strains used	Crown gall	Hairy root	Bacillus radiobacter	
Crown gall.....	3	+	0	0	
	1	0	0	0	
	1	0	a +	0	
Hairy root.....	10	0	+	0	
	1	0	0	0	
Bacillus radiobacter.....	2	0	0	+	
	1	0	0	0	

* In these tests and also in litmus milk, glycerophosphate media, and in the fermentation of xylose, rhamnose, galactose, mannose, lactose, and salicin, the behavior of this culture indicates a mixture of the crown-gall and hairy-root organisms.

STUDIES ON THE PROGENY OF SINGLE CELLS

An examination of the results secured with the cultures purified by four successive replatings (Table 3) shows that some of the original cultures were mixed. It also appears that even after the unusual precautions taken to purify these cultures by the poured-plate method, 2 of the 15 crown-gall and hairy-root strains (T-35-1a and T-38-1a) employed for bacteriological studies were mixed cultures. In the bacteriological studies and the inoculation work, the responses of each of these strains were in part typical of the crown-gall organism and in part typical of the hairy-root organism. For some time it was considered that both of these strains might be pure cultures which varied from the usual type and were perhaps intermediate between the typical crown-gall and hairy-root organisms, as they showed mixed characters. In order to gain further evidence on this point, single-cell cultures were isolated, according to the methods of Wright and McCoy (32) and Wright and Nakajima (33) from strain T-38-1a and subjected to bacteriological studies and inoculation tests. The characters of this strain were somewhat less clearly those that might be expected from a mixed culture of the crown-gall and hairy-root organisms than were those of strain T-35-1a. As time was not available to analyze both strains, T-38-1a was chosen as seemingly the more perplexing one. Four single-cell cultures were isolated. One of these consistently showed the typical characters of the crown-gall organism, while all the others consistently showed the typical characters of the hairy-root organism. A more detailed account of this work is reported by Wright, Hendrickson, and Riker (34).

Even when the single-cell technic is used, certain questions may be raised concerning the quality and purity of the cultures.

(1) When only an occasional isolated cell would grow, as was the case in the earlier stages of development of the technic, it was questionable whether the few cells that grew were satisfactorily representative of the large majority that did not. The improvement of the technic to the point where the majority of the isolated cells grow appears to minimize any element of doubt that these cells may be regarded as satisfactory random samples of the population from which they are taken.

(2) The possibility that an unrecognized or ultramicroscopic stage of another microorganism might be carried along with the single cells deserves consideration. However, this possibility was greatly reduced by selecting the single cells from media at a time when growth conditions were most favorable for the development of the usual vegetative forms.

(3) Attention should also be given the further possibility that some ultramicroscopic form of life might be transferred along with the single cells. All bacterial cultures are subject to this question, and it appears that they must continue to be unless biological science develops methods that will completely insure the detection of any living thing which may exist as a contaminant. In view of the lack of such technic at present, it seems desirable to seek to apply the best available methods to the purification of cultures and to evaluate the probability of their purity as critically as possible.

Apart from the technic of isolation, the best available criterion of the purity of single-cell cultures appears to be their behavior. If this is erratic, it remains questionable whether the variability results from genetic plasticity of the strain or from impurity of the culture. If, on the other hand, there is unusual consistency in the behavior of the strain throughout a sufficiently exacting morphological and physiological study, the chances of contamination or admixture appear to be minimized. Under the conditions thus far employed, the single-cell strains studied have shown a striking consistency in behavior. It remains for further work to reveal their behavior under diverse conditions. This technic seems to offer a satisfactory basis upon which to study variability of the organisms under investigation. In view of the extreme difficulty experienced in consistently separating the crown-gall and hairy-root organisms from each other and from various similar bacteria, it would appear that any report of variability within this group of organisms which is not based upon single-cell isolations is open to question. The work on the present writers' cultures with the single-cell technic has been reported by Wright, Hendrickson, and Riker (34). Eight strains of single-cell origin from the crown-gall organism and nine of like origin from the hairy-root organism were studied. No single-cell strain showed mixed characters of the hairy-root and the crown-gall organisms. In every case the crown-gall organism has given the typical crown-gall reactions and the hairy-root organism the typical hairy-root reactions. While variations may very possibly be found by study of a larger number of cultures, those studied thus far have behaved very consistently.

IDENTITY AND TECHNICAL DESCRIPTION OF THE HAIRY-ROOT ORGANISM

The differential characteristics of *Phytomonas tumefaciens*, of the hairy-root organism, and of *Bacillus radiobacter* are shown in Table 8. The considerable number of characters which distinguish the hairy-root organism from the crown-gall pathogene shows that the two organisms are different. While the definition of a bacterial species is so much a matter of debate, the present writers hesitate to describe a new species. However, convenience, which is a primary requisite of a workable classification, demands that the hairy-root organism be given some designation. If it is considered a variety of the crown-gall organism and the classification is based chiefly on morphological and cultural characters, the difficulty arises that *B. radiobacter* and probably a number of other mucoid soil organisms should be included in the same species. If this were done, all these organisms, including those that cause crown gall and hairy root, would seem to be referable to *B. radiobacter*, this organism having been described prior to the others. The objections to discarding such a well-known and firmly established species as *Phytomonas tumefaciens* seem quite obvious. If, on the other hand, the responses of plants to inoculations are regarded as differential characters of specific rank, it would seem unnecessary to refer the crown-gall organism to *B. radiobacter*, and the crown-gall and hairy-root organisms and *B. radiobacter* would fall into three species.

TABLE 8.—Differential characteristics of *Phytophthora tumefaciens*, the hairy-root organism, and *Bacillus radiobacter*

Differential characters *	<i>P. tumefaciens</i> ^b (5 strains)	Hairy-root organism (10 strains)	<i>B. radiobacter</i> (3 strains)
Reaction on:			
Apple.....	Causes crown gall.....	Causes hairy root.....	No reaction.
Tomato.....	do.....	Slight or no reaction.....	Do.
Motility.....	Motility doubtful.....	Motile.....	Actively motile.
Growth on calcium glycerophosphate medium with mannitol.	Abundant.....	None to trace.....	Abundant, with brown pigment.
Action on litmus milk.....	(Deep serum zone..... Grayish brown..... Neutral.....)	(Slight serum zone..... Litmus reduced..... Acid.....)	(Deep serum zone..... Grayish brown..... Neutral.....)
Absorption of:			
Congo red.....	Strong.....	Slight.....	Very slight.
Brom-thymol blue.....	Slight.....	do.....	Strong.
Nitrate reduction.....	No nitrite.....	No nitrite.....	Nitrate disappears.
Action on:			
Arabinose and xylose.....	(Slightly acid..... Strong fermentation *.....)	(Strongly acid..... Strong fermentation *.....)	(Slightly acid..... Strong fermentation.*.....)
Glucose.....	(Slightly acid..... Medium fermentation *.....)	(Strongly acid..... Weak fermentation *.....)	(Slightly acid..... Strong fermentation.*.....)
Galactose, fructose, and mannose.....	(Slightly acid..... Medium to strong fermentation.*.....)	(Strongly acid..... Medium to strong fermentation.*.....)	(Slightly acid..... Medium to strong fermentation.*.....)
Lactose.....	(No acid..... Weak fermentation *.....)	(Strongly acid..... Medium fermentation *.....)	(No acid..... Weak fermentation.*.....)
Maltose.....	(No acid..... Medium fermentation *.....)	(Slightly acid..... Medium fermentation *.....)	(Slightly acid..... Medium fermentation.*.....)
Salicin.....	Slightly acid.....	Strongly acid.....	No acid.
Erythritol.....	Slightly alkaline.....	do.....	Slightly alkaline.
Agglutination.....	In own antiserum only *.....	In own antiserum only *.....	In own antiserum only.*.....

* All strains of each group gave the same result unless otherwise noted.

^b Two of the five strains used throughout this work were secured from single-cell isolations. The reactions of T-35-1a, which is not a single-cell strain, are not included in this summary because this culture appears to be a mixture of crown-gall and hairy-root organisms.

* Amount of sugar fermented was ascertained by quantitative sugar determinations.

* Exceptions are noted in Table 7.

In view of the very considerable differences in bacteriological characters and host responses found between the hairy-root and crown-gall organisms, and the ample precedents for making a new species of an organism which is so distinct in a number of characters, it appears more useful, in the opinion of the present writers, to treat the hairy-root organism as a new species. The hairy-root organism, as described in detail in an earlier section of this paper, is therefore named *Phytophthora rhizogenes*, n. sp. Synonyms according to other systems of classification in common use among plant pathologists are *Bacterium rhizogenes*, n. sp. and *Pseudomonas rhizogenes*, n. sp. A brief description follows.

Phytophthora rhizogenes, n. sp.

Short nonspore-forming rods, 0.55–2.59 μ by 0.15–0.75 μ , average 1.44 μ by 0.43 μ ; motile by means of one polar flagellum; capsule present; Gram-negative; not acid-fast. On clear potato-glucose agar at 25° C. for seven days the surface colonies have these characters: Growth rapid; form circular, 2 to 6 mm. in diameter; surface smooth; elevation convex; edge smooth; internal structure finely granular; optical characters, translucent through gray to almost white. Deep colonies are lenticular and dense. Little or no growth in glycerophosphate mannitol medium, liquid or solid, with potassium nitrate as nitrogen source. Acid is produced in litmus milk; the litmus is slowly reduced, and a slight serum zone is formed which later disappears. Watery, moderate to scant growth on potato plugs. No growth below pH 4.0. Absorbs Congo red and brom-thymol blue slightly. No nitrite formed from nitrate. No apparent growth in the closed arm of fermentation tubes. Acid but no gas from arabinose, xylose, rhamnose, glucose, galactose, mannose, maltose, lactose, salicin, and erythritol. No acid or gas from fructose, sucrose, raffinose, melezitose, starch, dextrin,

inulin, aesculin, dulcitol, and mannitol. Starch not hydrolyzed. Favorable temperature for growth, 20° to 28° C. Causes infectious hairy root on apple.

SUMMARY

An infectious type of hairy root on nursery apple trees is described.

A limited number of bacteriological examinations of burrknots have failed to reveal any causal organism.

Isolation studies from 96 enlargements of the hairy-root type, including "woolly knot," are recorded. This number includes the cultures employed in preliminary work. From these, 367 cultures were used for inoculation studies, which involved 4,252 inoculations into tomato, tobacco, and apple. Four of the 96 enlargements were found to contain the crown-gall organism, 1 yielded cultures that were apparently mixtures of the crown-gall and hairy-root organisms, 13 yielded no pathogenic organisms, and 78 yielded the hairy-root organism.

Attempts were made to reisolate the hairy-root organism from 40 hairy-root specimens induced by inoculation. All yielded typical cultures. Cultures from 12 of these were reinoculated into apple. Typical hairy root was induced by bacteria from each of these 12 specimens.

The hairy-root organism has been inoculated into stems of apple, rose, honeysuckle, sugar beet, bean, and Paris daisy with positive results. Little or no response was induced in stems of tomato and tobacco.

In preliminary experiments cuttings or layers of certain plants treated with the hairy-root organism have rooted sooner and more vigorously than those untreated. These results suggest the possibility of using this organism to stimulate root production in the propagation of certain plants. More work is necessary before conclusions can be drawn.

The roots stimulated by the hairy-root organism have been found capable of supporting the life of apple, Delphinium, and Paris daisy plants after all the other roots were removed. Whether the effects of the hairy-root organism on the several plants over a long period are harmful or beneficial has not yet been determined.

The reactions of the underground parts of Wealthy apple stems to crown-gall inoculations, hairy-root inoculations, and needle punctures were very similar at the end of two weeks in preliminary comparative trials in Wisconsin. After a month the enlargements showed a decided increase in size and a change in surface character to the crown-gall type, while those of the controls were very little larger and showed no change in character. After two months the symptoms of hairy root were beginning to differentiate from those of crown gall, though at this stage they still might easily be confused. The controls at this time had healed over. Typical clearly differentiated hairy-root and crown-gall symptoms developed within three months.

Many of the roots which followed hairy-root inoculation were winterkilled in Wisconsin, but new roots of like type appeared the following spring. In the second year after inoculation deeply convoluted knots were often found at the places of hairy-root inoculation. These knots need further investigation.

A detailed comparative study was made of the bacteriological characters of 5 strains of *Phytomonas tumefaciens*, 10 strains of the

hairy-root organism, and 3 strains of *Bacillus radiobacter*. Their differences are summarized in Table 8.

The poured-plate method, even when repeatedly employed in successive trials and with special precautions, was found inadequate consistently to separate the crown-gall and hairy-root organisms from each other.

The bacteriological studies reported on the crown-gall and hairy-root organisms have been checked up and extended by studies reported elsewhere (34) on cultures which were progenies of single-cell isolations. Confirmatory results were secured.

Typical crown-gall and hairy-root cultures have been separated by the single-cell isolation technic from a culture that was earlier thought a probable variant and intermediate form.

Because of the difficulty of separating the crown-gall and hairy-root organisms, reports of variability within these species appear to be open to question when they do not deal with progeny from single-cell isolations.

The hairy-root organism is described under the name *Phytomonas rhizogenes*, n. sp.

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STUDIES ON THE PROGENY OF SINGLE-CELL ISOLATIONS FROM THE HAIRY-ROOT AND CROWN-GALL ORGANISMS¹

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INTRODUCTION

The difficulty of separating uncontaminated strains of the crown-gall³ and hairy-root⁴ organisms and of *Bacillus radiobacter* Beij. and Van Deld.⁵ has been discussed by Riker and others.⁶ These writers reported that the poured-plate method was inadequate to separate the organisms of this group into pure cultures with certainty. They reached this conclusion after taking unusual care in preparing the dilutions, in pouring the dilution plates, and in making four successive isolation series from each of 31 original cultures of the crown-gall and hairy-root organisms. Some of the reasons for the difficulty in obtaining pure strains of these bacteria by the poured-plate method are (1) the mixture of organisms, including various soil bacteria, that commonly occurs in the underground parts from which the original platings are made; (2) the large amount of slime produced by the bacteria, which hinders separation of individual cells; and (3) the fact that isolated single cells ordinarily fail to grow when placed in a comparatively large quantity of medium. In repeated trials by the present writers less than 1 per cent of the single cells of these organisms grew when each was placed in 10 c. c. of sterile medium of the most favorable kind known. Consequently, when a cell becomes well separated from others in a poured-agar plate, the chances in favor of its growth seem very small. For a long time this inability to get the isolated cells to grow prevented the successful use of the single-cell technic for studies of *Phytophthora tumefaciens* and *P. rhizogenes*.

SINGLE-CELL ISOLATIONS

The improvement of the single-cell technic by Wright and McCoy⁷ and later by Wright and Nakajima⁸ provided a new and practical

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² Professor Wright died May 3, 1929, when the work was almost completed. The few remaining experiments were performed and the manuscript prepared as nearly as possible in the way that he had planned.

³ *Phytophthora tumefaciens* (Smith and Town.) Com. S. A. B.

⁴ *Phytophthora rhizogenes* R., B., W., K., and S.

⁵ BEIJERINCK, W. M., and DELDEN, A. VAN. UEBER DIE ASSIMILATION DES FREIEN STICKSTOFFE DURCH BAKTERIEN. Centbl. Bakt. [etc.] [II] 9: 3-43. 1902.

⁶ RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., KEITT, G. W., and SAGEN, H. E. STUDIES ON INFECTIOUS HAIRY ROOT OF NURSERY APPLE TREES. Jour. Agr. Research 41: 507-540, illus. 1927.

⁷ WRIGHT, W. H., and MCCOY, E. F. AN ACCESSORY TO THE CHAMBERS APPARATUS FOR THE ISOLATION OF SINGLE BACTERIAL CELLS. Jour. Lab. and Clin. Med. 12: 795-800, illus. 1927.

⁸ WRIGHT, W. H., and NAKAJIMA, H. THE GROWING OF PURE CULTURES FROM SINGLE CELLS OF NON-SPORE FORMING BACTERIA. (Abstract) Jour. Bact. 17: 10-11. 1929.

method of studying the crown-gall and hairy-root organisms from cultures that were the progeny of single cells. Consequently, some of the cultures employed by Riker and his coworkers,⁹ were used for making single-cell isolations, as mentioned earlier. Since the same culture numbers adopted by these writers are used here, the reader is referred to their paper for the histories and the records of pathogenicity of the original cultures. The original cultures employed for single-cell isolations were as follows: Crown gall, T-5; intermediate (?) between hairy root and crown gall, T-38; and hairy root, T-32 and T-48.

The medium which seemed most suitable for the single-cell isolation work was selected after trials with a number of different media. The peptone-salt medium selected as the best had the following composition: Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.2 gm.; sodium chloride (NaCl), 0.2 gm.; dipotassium phosphate (K_2HPO_4), 0.2 gm.; calcium chloride (CaCl_2), 0.1 gm.; peptone, 5 gm.; and distilled water 1,000 c. c. The reaction was pH 7.0.

The age of the culture at the time the single cells were picked was very important. Since cultures that are too old or too young may contain a number of organisms that are either dead or not actively growing, it was necessary to determine for each strain the time most favorable for finding rapidly dividing cells, or the "logarithmic growth phase" as defined by Buchanan.¹⁰ This was accomplished by making counts on the cultures used, according to the method of Breed and Brew.¹¹ The first count was made immediately after the transfer. Subsequent counts were made after 6, 10, 14, 18, 22, and 26 hours, respectively, at 21° C. Graphs of the results of these counts are given in Figure 1. The curves show that under the conditions employed the bacteria of all the cultures were in the "logarithmic growth phase" during the period between 12 and 16 hours after transfer. Consequently this time was chosen for making the single-cell isolations.

The methods employed in isolating and cultivating the single bacterial cells were modifications of Chambers's technic as described by Wright and McCoy¹² and Wright and Nakajima.¹³ Part of this modified technic is briefly as follows: Two sterile cover slips served as covers for the glass moist chamber used on the stage of the microscope. One of these carried a small drop of the culture of the desired age and the other a small drop of sterile medium. Two sterile micro-pipettes, with apertures approximating three times the length of the organisms to be isolated, were alternately employed in making the isolations. The edge of the drop containing the organisms was placed in the field of the microscope by moving the mechanical stage. One of the pipettes was made to touch the edge of this drop, a portion of which was drawn into the pipette by capillarity. The pipette and cover slip were manipulated so as to deposit a series of microscopic drops on the dry portion of the cover slip. The process was continued until a microscopic drop formed that was observed to contain only one cell. This microscopic drop with the single organism was then

⁹ See footnote 6.

¹⁰ BUCHANAN, R. E. LIFE PHASES IN A BACTERIAL CULTURE. *Jour. Infect. Diseases* 23: [109]-125, illus. 1918.

¹¹ BREED, R. S., and BREW, F. D. COUNTING BACTERIA BY MEANS OF THE MICROSCOPE. *N. Y. State Agr. Expt. Sta. Tech. Bul.* 49, 31 p., illus. 1916.

¹² See footnote 7.

¹³ See footnote 8.

picked up with the other sterile micropipette and deposited on the other coverslip near the small drop of sterile medium. The microscopic drop was then examined to make certain that the one cell, and only that one, was present. With the rubber-tube attachment to

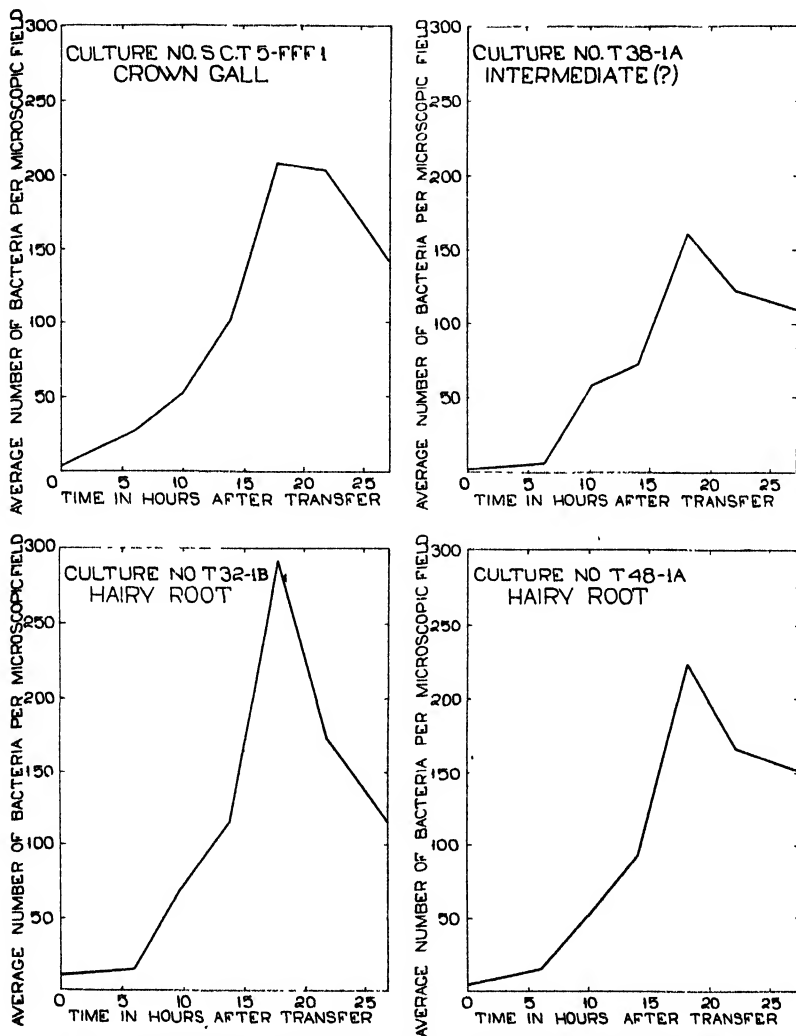


FIGURE 1.—Results secured from direct counts of the number of bacteria present in the peptone-salt medium at progressive intervals of time following transfers from the original cultures employed

the pipette the edge of the small sterile drop was blown gently around the microscopic drop containing the single cell until the two drops merged. The cover slip with the single cell in the small drop of fresh medium was removed from the moist chamber on the microscope and mounted with sterile vaseline on a sterile hanging-drop slide. A drop of sterile medium was provided at the bottom of the depression in the

slide to prevent drying. The slides prepared in this manner were incubated at about 21° C. After two to five days, when the drop showed bacterial growth, it was increased in size by adding sterile medium from a capillary pipette. By gradually increasing the amount of medium in two or three increments at suitable intervals a condition was reached which permitted transfer of the culture to a liquid or solid medium in a test tube, without fear of killing the organisms.

Usually a high percentage (up to 90 per cent in the best runs) of the single cells isolated from the crown-gall and hairy-root organisms by this technic grew. Thus far no case of contamination has been observed. This method insures the purity of the cultures as far as the present available technic permits. Although the possibility remains that the ultramicroscopic form of some other organism might be carried along with the single cell, this seems quite remote because (1) the cultures from which the cells were picked were provided with favorable conditions for the development and active growth of the microscopic forms and (2) subsequent bacteriological studies of the progenies of the single cells did not give evidence of mixture. There is also the possibility that a filterable virus might be carried along with the single cell. This possibility has been discussed by Riker and his associates¹⁴ and will not be considered here.

With the single-cell technic described, several series of isolations were made of the typical crown-gall and hairy-root organisms. From the crown-gall culture T-5-fff1 (subculture of T-5) the following cultures were isolated: A-1, A-2, A-3, A-4, A-5, A-6, and A-7; from the culture T-38-1a (subculture of T-38)—a culture intermediate (?) between hairy root and crown gall—B-2, B-3, B-4, and B-5; from the hairy-root culture T-32-1b (subculture of T-32), C-1, C-10, C-11, C-12, and C-13; and from the hairy-root culture T-48-1a (subculture of T-48), D-20. Culture T-5-fff1 was itself of single-cell origin from T-5. Each of the other cultures from which the single cells were isolated had been replated four successive times from the original culture. The identifications of the cultures were based on inoculations on tomato and apple and on bacteriological studies, as described earlier.¹⁴ T-38-1a, the intermediate (?) culture, produced crown gall on tomato and a mixture of crown gall and hairy root on apple. However, its bacteriological reactions were predominantly those of the hairy-root organism.

BACTERIOLOGICAL STUDIES

The bacteriological studies carried out on these single-cell cultures and their mother cultures were in each case parallel to those reported by Riker and his associates.¹⁴ Since the present writers used the technic which these authors have fully described in their paper, only the results are recorded here.

Morphologically all the crown-gall and hairy-root cultures were small rods, approximately 0.3μ to 0.4μ by 0.5μ to 3.0μ . They were nonspore-forming, Gram-negative, and not acid-fast. Limited experiments showed that the hairy-root organism was motile by one polar flagellum. Experiments thus far conducted with the crown-gall organism have shown no flagella and no motility.

Absorption of Congo red was shown by all the cultures on the yeast-infusion glucose agar after 11 days at room temperature.

¹⁴ See footnote 6.

However, the absorption by the crown-gall organism was, on an average, somewhat stronger than that by the hairy-root organism.

Indol was not produced, starch was not hydrolyzed, and nitrate was not reduced to nitrite by any of the crown-gall or hairy-root cultures.

The cultural characteristics of the crown-gall organism were markedly different from those of the hairy-root organism in certain media. Differentiation was secured in calcium glycerophosphate agar with mannitol. All the crown-gall cultures grew abundantly, while the hairy-root organism consistently made little or no growth. In liquid peptone and in liquid yeast-infusion glucose media the crown-gall cultures, without exception, produced a pellicle, a veil, and a slight precipitate, but no turbidity. In the same media none of the hairy-root cultures produced a pellicle or a veil, but all showed either moderate or abundant clouding with a slight precipitate. These cultures were incubated for six days at 30° C. Litmus milk also differentiated these two groups of organisms. The crown-gall organism caused this medium to turn alkaline, reduced the litmus, and produced a conspicuous serum zone at the top. The hairy-root organism produced acid, reduced the litmus, and formed a serum zone which largely or entirely disappeared after three weeks at 30° C. These results are recorded in Table 1.

Inoculation tests with each of these organisms were made on the roots of sugar beets in the greenhouse. At the end of two months each of the crown-gall cultures had induced crown gall and each of the hairy-root cultures had induced hairy root. (Table 1.) Further inoculation studies are being made on other test plants; including apple.

The single-cell analysis of the culture T-38-1a, which was earlier regarded as possibly a pure culture intermediate between the crown-gall and hairy-root organisms because it had shown a mixture of the characters of these organisms in the bacteriological studies and inoculation work, has shown consistent results. In every test employed the single-cell progenies B-2, B-4, and B-5 from this culture have behaved like the hairy-root organism. On the other hand, the single-cell progeny B-3 has consistently behaved like the crown-gall organism. These organisms apparently had not been separated, even by the unusual care taken with the four successive poured-plate isolations. These results present strong evidence that, instead of being a pure culture intermediate between the crown-gall and hairy-root organisms, T-38-1a was a mixed culture of both.

Under the conditions and over the range of characters studied, the behavior of the seven single-cell progenies, A-1, A-2, A-3, A-4, A-5, A-6, and A-7, from the crown-gall stock culture T-5-fff1, was remarkably consistent. These studies are being continued to gain further evidence concerning the range of variability of the crown-gall and hairy-root organisms.

The fermentation of carbohydrates showed a distinct difference between the crown-gall and hairy-root organisms. Determinations were made of the actions of these organisms on 0.5 per cent solutions of xylose, glucose, galactose, mannose, lactose, and erythritol in the liquid peptone medium as measured by the hydrogen-ion concentration. From each of these carbohydrates each of the hairy-root cultures produced a considerable amount of acid, while each of the crown-gall cultures produced little or no acid. The results of this work are given in Table 2.

TABLE 1.—Growth characters in different media and reactions on sugar beets shown by single-cell strains of the crown-gall and hairy-root organisms and by their mother cultures^a

Culture used	Mother culture	Characters in—										Reaction on sugar beet
		Calcium glyco-phosphate agar with mannitol ^b	Liquid peptone medium ^c		Liquid yeast-infusion glucose medium ^d		Litmus milk			Serum zone		
			Growth	Pellicle	Veil	Turbidity	Pellicle	Veil	Turbidity		Acid	
Crown gall T-5-ffl.	T-5-ffl.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Crown gall
Crown gall A-1	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Crown gall A-2	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Crown gall A-3	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Crown gall A-4	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Crown gall A-5	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Crown gall A-6	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Crown gall A-7	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Do.
Intermediate (?) T-38-la	T-38	0	0	0	+++	0	0	+++	+++	0	0	Hairy root
Hairy root B-2	T-38-la	++	++	+	0	+++	++	0	+++	0	++	Do.
Crown gall B-3	do.	+++	+++	+++	0	+++	+++	0	0	+++	+++	Crown gall
Hairy root B-4	do.	0	0	0	0	0	0	0	0	0	0	Hairy root
Hairy root B-5	do.	0	0	0	+++	0	0	+++	+++	0	++	Do.
Hairy root T-32-lb	T-32	0	0	0	+++	0	0	+++	+++	0	++	Do.
Hairy root C-1	T-32-lb	+	+	+	0	0	0	0	0	+	+	Do.
Hairy root C-10	do.	0	0	0	0	0	0	0	0	0	0	Do.
Hairy root C-11	do.	0	0	0	0	0	0	0	0	0	0	Do.
Hairy root C-12	do.	0	0	0	0	0	0	0	0	0	0	Do.
Hairy root C-13	do.	0	0	0	0	0	0	0	0	0	0	Do.
Hairy root T-48-la	T-48	++	++	0	0	0	0	0	0	0	0	Hairy root.
Hairy root D-20	T-48-la	++	++	0	0	0	0	0	0	0	0	Do.

^a The different degrees in the development of the various characters are shown as follows: 0, none; +, trace; ++, moderate; and ++++, abundant.^b This medium contained: Mannitol, 20 gm.; potassium nitrate (KNO₃), 5 gm.; sodium chloride (NaCl), 3.8 gm.; potassium chloride (KCl), 0.1 gm.; magnesium chloride (MgCl₂·6H₂O), 1 gm.; magnesium sulphate (MgSO₄·7H₂O), 0.9 gm.; calcium glycerophosphate Ca (C₃H₅(OH)₂·HPO₄), 0.8 gm.; agar 15 gm.; and water, 1,000 c. c. The reaction was pH 6.8. The agar had been washed for two days at 37° C.^c This liquid medium contained: Yeast, 5 gm.; peptone, 5 gm.; and distilled water, 1,000 c. c. The reaction was pH 7.0.^d This medium contained: Glucose, 5 gm.; magnesium sulphate (MgSO₄·7H₂O), 0.2 gm.; sodium chloride (NaCl), 0.2 gm.; dipotassium phosphate (K₂HPO₄), 0.2 gm.; calcium chloride (CaCl₂), 0.1 gm.; and water, 1,000 c. c. The reaction was pH 7.0.

TABLE 2.—Acid production from different carbohydrates by single-cell strains of the crown-gall and hairy-root organisms and by their mother cultures ^a

Culture used	Mother culture	Acid production from—					
		Xylose	Glucose	Man- nose	Galac- tose	Lactose	Eryth- ritol
		pH	pH	pH	pH	pH	pH
Crown gall T-5-ffff1	T 5	5.8	5.6	6.0	5.4	5.8	6.8
Crown gall A-1	T 5-ffff1	5.8	5.9	6.2	5.0	6.8	6.8
Crown gall A-2	do.	5.9	6.0	6.1	5.0	6.8	6.8
Crown gall A-3	do.	6.0	6.0	6.0	5.1	6.8	6.8
Crown gall A-4	do.	6.2	5.8	6.0	5.0	6.7	6.8
Crown gall A-5	do.	6.2	6.0	6.2	5.2	6.6	6.8
Crown gall A-6	do.	5.9	6.8	6.1	5.1	6.2	6.8
Crown gall A-7	do.	5.8	5.6	6.2	5.0	6.7	6.8
Intermediate (?) T-38-1a	T-38	4.6	4.4	4.0	4.3	4.8	5.1
Hairy root B-2	T-38-1a	4.7	1.8	4.4	4.6	4.6	4.9
Crown gall B-3	do.	5.8	5.8	6.4	5.0	6.8	6.8
Hairy root B-4	do.				4.2	1.4	5.9
Hairy root T-32-1b	T-32	4.7	4.7	4.8	4.3	4.5	5.1
Hairy root C-1	T-32 1b	4.4	4.8	4.5	4.2	4.8	5.0
Hairy root C-10	do.	4.8	4.8	4.9	4.4	4.8	4.9
Hairy root C-11	do.	4.1	4.3	4.2	1.3	4.4	4.7
Hairy root C-12	do.	4.9	4.9	4.7	1.3	4.8	5.0
Hairy root C-13	do.	5.0	4.8	4.9	4.4	4.5	5.0
Hairy root T-48-1a	T 48	4.7	4.7	4.5	4.4	4.8	4.8
Hairy root D-20	T 48 1a	4.3	4.6	4.4	4.3	4.2	4.6
Control		6.8	6.8	6.8	6.8	6.8	6.8

^a The readings were taken after 12 days at 30° C. The sugars were sterilized separately and added aseptically to liquid peptone-salt medium. The formula is given in footnote c of Table 1.

SUMMARY

The poured-plate method is shown to be inadequate for the consistent isolation of uncontaminated cultures of the crown-gall and hairy-root organisms (*Phytoplasma tumefaciens* and *P. rhizogenes*).

Usually a high percentage of the single cells isolated from cultures of the crown-gall and hairy-root organisms have been made to grow.

The cultures from single-cell isolations were remarkably consistent in their behavior. Those from the crown-gall pathogene uniformly gave typical reactions for that organism. Likewise, those from the hairy-root organism consistently gave typical reactions for that organism. Four single-cell isolations were made from a culture that showed a mixture of the characters of the crown-gall and hairy-root organisms and was earlier thought to be possibly a pure culture of an organism intermediate between these. In the bacteriological studies and inoculation tests one of these single-cell strains consistently showed the typical characters of the crown-gall organism and the other three consistently showed those of the hairy-root organism.

In consideration of the limitations of the poured-plate method for purifying cultures, it appears that reports of variations of the crown-gall and hairy-root organisms not based upon work with single-cell isolations are open to question.

SIZE OF LIST QUADRAT FOR USE IN DETERMINING EFFECTS OF DIFFERENT SYSTEMS OF GRAZING UPON AGROPYRON SMITHII MIXED PRAIRIE¹

By HERBERT C. HANSON, formerly *Associate in Botany, Colorado Agricultural Experiment Station*² and L. DUDLEY LOVE, *Assistant in Botany, North Dakota Agricultural College*³

INTRODUCTION

The question of the most suitable size of quadrat to use in analyzing herbaceous vegetation is an important one. In studying the effects of two different systems of grazing upon the same kind of vegetation in adjacent range pastures it became necessary to adopt a standard size of quadrat.

A number of factors influenced the determination. One of the most important of these was the method of comparing the vegetation in the pastures. It was decided to use a rather large number of list quadrats distributed fairly uniformly even though at random over the areas compared. This method would yield data on the abundance and frequency of all species including the important forage plants and overgrazing indicators. To save time it was desirable to use as small areas as possible. The time factor was important because the period during which the early spring plants are still green and the late summer plants tall enough to be identified is rather short and also because of the expense involved. Other factors that influenced the size of the quadrat were the kind, density, and uniformity of the vegetation.

The two pastures under study are located on the plain at the base of the foothills at an elevation of about 5,000 feet near Fort Collins, Colo. The physical conditions and vegetation in these pastures have been briefly described by Hanson and Ball (6).⁴ In one pasture the deferred and rotation method of grazing had been practiced since 1921, in the other the continuous grazing method was used. (Fig. 1.) By the deferred and rotation method grazing was deferred for two years in succession on one half of the pasture until about August 15; on the other half meanwhile grazing was permitted from the time the range was ready in the spring, about May 1 to 7. At the end of two years the portion which had been grazed early was allowed to rest until about August 15 while the other half was grazed. Only adjacent portions of the pastures that were similar in relief and soil were studied in detail. Important differences in the vegetation, then, would appear to be due to the differences in the systems of grazing. The portion in the deferred and rotation pasture comprised about 72 acres and the portion in the other pasture comprised about 63 acres.

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³ The authors acknowledge the assistance given them by Andrew Clark, associate professor of mathematics, Colorado Agricultural College, who suggested the statistical methods that were used to analyze the data on abundance and frequency.

⁴ Reference is made by number (italics) to Literature Cited. p. 560.

METHODS

The method employed to locate the quadrats was to lay out three lines equidistant from each other and parallel with the width of the pasture. One of these lines was near the middle of the area under study, the other two toward the ends. On each of these lines 10 points for quadrat studies were located at intervals of 125 feet (38 meters) in the deferred and rotation pasture and at intervals of 80 feet (24 meters) in the other.

The following sizes of quadrats were used at each of the 10 points on each line: 0.25, 0.5, 1, 2, 3, and 4 square meters. The area of 4 square meters included all of the other units. The total number of quadrats of each size in each pasture was 30. For each quadrat the species of plants were listed and tabulated. Whenever possible the individual stalks, as in *Agropyron*, *Psoralea*, and *Artemisia*, were counted. In the case of sod formers, such as *Bouteloua* and *Bulbilis*, descriptive terms were used to denote the abundance. The frequency

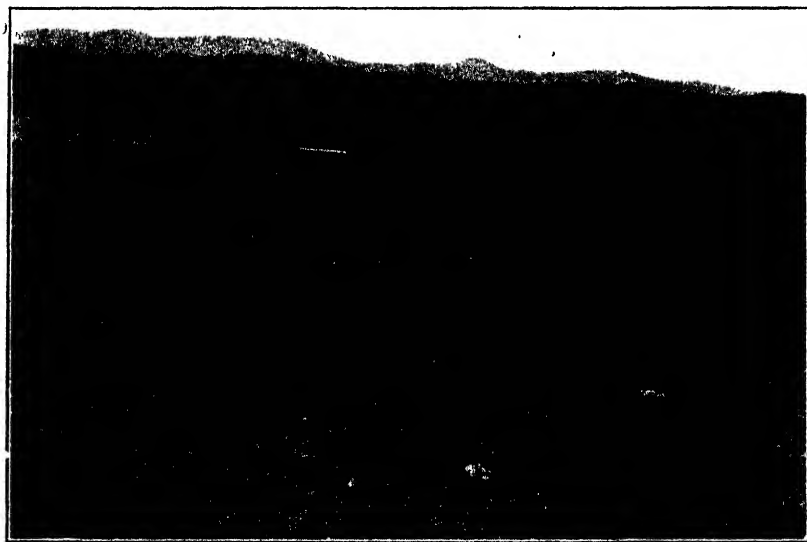


FIGURE 1.—View in the continuously grazed pasture showing the type of vegetation. *Agropyron smithii* and *Senecio perplezus* are prominent. May 21, 1929

and abundance of each species for each size of quadrat were determined (Tables 1 and 2) and the species were grouped on the basis of grazing value for cattle into three classes—desirable, undesirable, and immaterial. The desirable species are those that furnish considerable forage or that are highly palatable but because of their scarcity furnish little feed. The undesirable species are those that are poisonous, mechanically injurious, or that are not grazed and in addition make heavy demands upon essential factors such as soil moisture. Often they are strong and aggressive competitors. The immaterial species are those that are grazed but slightly, and often perhaps accidentally with other plants, or those that are not grazed and, moreover, appear not to make heavy demands upon the habitat, nor do they appear to be aggressive competitors.

TABLE 1.—Frequency and abundance of species occurring in 30 quadrats of six different sizes in the deferred and rotation pasture, June 11–28, 1929*

[The total figures of each size of quadrat are included in the next larger size]

Species	0.25 square meter quadrat		0.5 square meter quadrat		1 square meter quadrat		2 square meter quadrat		3 square meter quadrat		4 square meter quadrat	
	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance
	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number
Desirable species												
<i>Agropyron Smithii</i> Rydb.	97	4,210	100	8,120	100	16,281	100	27,460	100	41,762	100	53,884
<i>Schedonnardus paniculatus</i> (Nutt.) Trel.	67	8	73	8	83	8	90	1	90	1	90	1
<i>Senecio perplexus</i> A. Nels.	47	81	57	161	63	335	70	501	77	875	80	1,148
<i>Eurotia lanata</i> (Pursh) Moq.	27	16	50	29	60	46	70	69	73	98	73	124
<i>Astragalus drummondii</i> Dougl.	23	21	30	31	40	45	60	113	67	163	70	197
<i>Bouteloua gracilis</i> (H. B. K.) Lag.	27	5	34	5	47	1	47	1	57	1	57	1
<i>Aristida longiseta</i> Steud.	30	5	30	5	30	5	37	5	43	5	47	5
<i>Helianthus pumilus</i> Nutt.	10	6	17	10	27	20	30	51	37	66	47	87
<i>Stipa viridula</i> Trin.	13	13	17	25	17	53	20	110	20	168	20	229
<i>Vicia linearis</i> (Nutt.) Greene.	4	5	3	7	3	14	3	24	3	38	3	50
<i>Carex stenophylla</i> Wahl.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trifolium repens</i> L.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Poa</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
Undesirable species												
<i>Gutierrezia longifolia</i> (Greene) K.	37	14	50	25	57	42	77	75	80	100	87	132
<i>Iva axillaris</i> Pursh.	43	99	53	175	63	370	67	716	73	1,023	73	1,381
<i>Psoralea tenuiflora</i> Pursh.	47	28	50	48	57	94	63	165	70	220	73	283
<i>Linum Lewisii</i> Pursh.	13	12	13	15	30	70	17	103	47	119	50	186
<i>Artemisia gnaphalodes</i> Nutt.	33	34	40	69	40	122	43	278	50	503	50	608
<i>Bahia oppositifolia</i> Nutt.	17	15	30	29	33	58	40	138	47	193	53	331
<i>Bulbils dactyloides</i> (Nutt.) Raf.	13	V	17	V	23	N	37	1	43	1	53	1
<i>Helianthus annuus</i> L.	3	1	17	8	23	14	33	31	43	57	47	72
<i>Opuntia humifusa</i> Raf.	0	0	7	1	13	1	23	8	27	13	37	19
<i>Quercula lobata</i> (Torr.) Raf.	7	4	10	10	10	12	20	22	23	52	23	81
<i>Muhlenbergia gracillima</i> Torr.	13	V	13	V	17	N	17	8	20	8	20	8
<i>Yucca glauca</i> Nutt.	0	0	7	2	7	2	17	9	20	10	20	13
<i>Artemisia frigida</i> Willd.	3	4	3	4	7	5	17	12	23	14	30	19
<i>Aster hebecladus</i> DC.	7	19	10	58	10	104	13	282	13	365	17	513
<i>Artemisia dracunculoides</i> Pursh.	0	0	7	5	10	8	13	17	13	19	20	28
<i>Argemone intermedia</i> Sweet.	0	0	3	1	10	9	10	9	10	9	10	10
<i>Sitanion brevifolium</i> J. G. Smith.	3	12	7	19	7	19	10	22	13	25	17	30
<i>Hordeum nodosum</i> L.	0	0	0	0	3	1	7	2	7	3	7	3
<i>Cryptantha crassispala</i> (T. and G.) Greene.	3	3	3	3	7	6	7	10	10	14	10	21
<i>Grindelia squarrosa</i> (Pursh) Dunal.	0	0	0	0	3	1	3	1	3	3	3	3
<i>Nothocalais cuspidata</i> (Pursh) Greene.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bromus tectorum</i> L.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asclepias pumila</i> (Gray) Vail.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euphorbia marginata</i> Pursh.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Astragalus bisulcatus</i> (Hook.) Gray.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sporobolus cryptandrus</i> (Torr.) Gray.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stanleya pinnata</i> (Pursh) Brit.	7	2	7	5	7	5	7	9	10	12	10	16
<i>Carduus undulatus</i> Nutt.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mamillaria missouriensis</i> Sweet.	0	0	0	0	0	0	0	0	0	0	3	1
<i>Stipa vaseyi</i> Scribn.	0	0	0	0	0	0	0	0	0	0	0	0

* The following abbreviations are used in Tables 1 and 2: V. S., very scarce; S., scarce; I., infrequent; F., frequent; Ab., abundant.

TABLE 1.—Frequency and abundance of species occurring in 30 quadrats of six different sizes in the deferred and rotation pasture, June 11–28, 1929—Contd.

Species	0.25 square meter quadrat		0.5 square meter quadrat		1 square meter quadrat		2 square meter quadrat		3 square meter quadrat		4 square meter quadrat	
	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance
	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number
Immaterial species—Contd.												
<i>Camelina sativa</i> Crantz.	0	0	0	0	0	0	0	0	0	0	0	3
<i>Townsendia exscapa</i> (Rich.) Por.	0	0	0	0	0	0	0	0	3	1	3	1
<i>Eriocoma cuspidata</i> Nutt.	0	0	0	0	0	0	0	0	3	1	3	1
<i>Erysimum asperillum</i> (Greene) Rydb.	0	0	0	0	0	0	0	0	3	2	3	2
<i>Scutellaria brittonii</i> Porter	0	0	0	0	0	0	0	0	3	3	7	10

TABLE 2.—Frequency and abundance of species occurring in 30 quadrats of six different sizes in the continuously grazed pasture, June 11–28, 1929

Species	0.25 square meter quadrat		0.5 square meter quadrat		1 square meter quadrat		2 square meter quadrat		3 square meter quadrat		4 square meter quadrat	
	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance
	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number
Desirable species.	100	2, 284	100	4, 777	100	9, 807	100	17, 921	100	27, 810	100	36, 942
Agropyron smithii	30	V. S.	43	V. S.	57	S	60	S	67	S	70	S
Schedonardus paniculatus	13	8	23	22	23	43	40	85	40	136	40	170
Senecio perplexus	0	0	0	0	0	0	0	0	0	0	0	0
Eurotia lanata	0	15	13	17	20	F 40	30	61	37	122	37	147
Astragalus drummondii	10	15	13	17	20	F 40	30	61	37	122	37	147
Bouteloua gracilis	67	S	73	S	73	F	73	S	83	Ab	87	F
Aristida longistea	43	S	50	S	57	F	64	S	67	S	77	F
Hellanthus pumilus	13	13	20	18	37	55	40	91	57	147	60	214
Stipa viridula	0	0	0	0	0	0	0	0	3	4	3	4
Vicia linearis	0	0	0	0	3	2	3	7	3	9	3	16
Carex stenophylla	0	0	0	0	0	0	3	241	3	560	3	828
Trifolium repens	0	0	0	0	3	1	3	1	3	1	3	1
Poa sp.	0	0	0	0	0	0	3	V. S.	3	V. S.	3	V. S.
Undesirable species												
Gutierrezia longifolia	23	8	37	15	53	25	73	48	83	68	87	86
Iva axillaris	37	97	37	180	43	370	43	606	47	821	50	1, 021
Psoralea tenuiflora	60	75	73	131	90	286	100	544	100	825	100	1, 099
Linum lewisii	17	13	20	31	30	62	33	107	37	135	40	159
Artemisia gnaphalodes	27	32	30	67	33	130	53	260	60	374	60	579
Bahia oppositifolia	20	67	23	105	33	166	37	275	60	392	60	412
Buiblis dactyloides	23	S	23	S	23	S	23	S	37	S	37	S
Hellanthus annuus	0	0	0	0	3	1	10	3	10	6	13	9
Opuntia humifusa	0	0	0	0	0	0	13	3	23	7	37	14
Quincula lobata	0	0	0	0	0	0	3	3	10	4	10	4
Muhlenbergia gracillima	3	V. S.	3	V. S.	3	V. S.	3	V. S.	3	V. S.	3	V. S.
Yucca glauca	0	0	0	0	7	2	20	8	23	13	27	1
Artemisia frigida	17	6	17	18	27	32	47	57	53	81	57	107
Aster hebecladus	17	63	17	91	23	121	37	193	40	240	43	367
Artemisia dracunculoides	0	0	10	3	20	6	27	9	30	13	37	16
Agermone intermedia	0	0	0	0	17	9	17	9	17	9	20	15
Sitanion brevifolium	7	3	13	9	17	17	27	34	37	48	40	41
Hordeum nodosum	0	0	0	0	3	2	10	20	10	20	10	29
Cryptantha crassisejala	0	0	0	0	0	0	0	0	0	0	0	2
Grindelia squarrosa	3	1	3	1	7	2	13	4	17	5	17	0
Nothocalais cuspidata	7	2	7	2	7	3	10	9	10	9	10	16
Bromus tectorum	7	7	7	7	7	10	17	35	28	75	27	90
Asclepias pumila	3	8	3	45	3	19	3	30	3	30	3	48
Euphorbia marginata	3	1	3	1	3	1	3	1	3	1	3	2
Astragalus bisulcatus	0	0	0	0	3	12	3	12	3	13	3	11
Sporobolus cryptandrus	3	V. S.	3	V. S.	3	V. S.	3	V. S.	3	V. S.	3	V. S.

TABLE 2.—Frequency and abundance of species occurring in 30 quadrats of six different sizes in the continuously grazed pasture, June 11–28, 1929—Continued

Species	0.25 square meter quadrat		0.5 square meter quadrat		1 square meter quadrat		2 square meter quadrat		3 square meter quadrat		4 square meter quadrat	
	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance
	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number
Undesirable species—Contd.												
<i>Stipa vaseyi</i>	0	0	0	0	0	0	0	0	3	1	3	1
<i>Carduus undulatus</i>	3	1	3	2	3	2	3	2	3	2	3	2
<i>Stanleya pinnata</i>	0	0	0	0	0	0	0	0	0	0	3	1
Immateral species.												
<i>Sophora sericea</i>	30	32	33	50	40	92	43	173	47	224	47	304
<i>Gaura coccinea</i>	43	28	57	53	80	115	93	194	97	297	97	398
<i>Musineon divaricatum</i>	87	121	90	230	93	405	97	771	100	1,204	100	1,546
<i>Malvastrum coccineum</i>	47	45	70	86	77	181	83	339	87	495	90	627
<i>Draba nemorosa</i>	37	167	47	282	57	554	63	958	67	1,454	71	1,774
<i>Sophia pinnata</i>	33	25	43	50	70	116	83	211	93	269	97	343
<i>Allium reticulatum</i>	7	4	10	6	10	6	30	16	30	18	33	25
<i>Viola nuttallii</i>	7	2	13	5	23	13	30	18	33	27	43	34
<i>Polygonum douglasii</i>	3	1	13	8	13	9	13	12	17	13	17	14
<i>Lepidium apetalum</i>	17	13	37	23	40	43	53	78	60	110	60	127
<i>Lappula occidentalis</i>	13	6	17	10	20	39	23	58	33	78	40	95
<i>Astragalus hypoglottis</i>	20	36	30	65	43	124	47	303	57	509	67	634
<i>Lithospermum angustifolium</i>	0	0	3	1	7	2	7	2	17	12	20	18
<i>Astragalus shortianus</i>	3	1	3	1	10	4	17	13	20	17	23	20
<i>Lactuca pulchella</i>	0	0	0	0	3	2	27	12	40	17	43	25
<i>Evolvulus pilosus</i>	17	24	23	34	33	89	50	194	60	300	67	372
<i>Allionia linearis</i>	13	6	13	7	20	11	33	24	43	35	50	43
<i>Petalostemon oligophyllus</i>	3	1	7	2	13	7	20	20	33	58	40	69
<i>Lesquerella montana</i>	3	1	7	2	7	6	10	11	20	18	27	27
<i>Plantago purshii</i>	10	6	17	10	20	19	23	38	33	73	37	91
<i>Taraxacum officinale</i>	0	0	3	1	3	1	7	3	10	5	13	7
<i>Eriogonum effusum</i>	3	1	7	2	20	9	27	15	37	31	40	38
<i>Androsace occidentalis</i>	3	1	3	3	7	10	7	10	7	40	7	40
<i>Liatris punctata</i>	13	6	27	26	37	54	43	89	57	161	63	217
<i>Astragalus flexuosus</i>	20	22	27	27	27	60	33	104	40	188	43	222
<i>Astragalus missouriensis</i>	20	11	30	14	43	26	60	67	73	105	73	128
<i>Sideranthus spinulosus</i>	10	1	10	3	13	6	13	6	30	14	33	18
<i>Euphorbia robusta</i>	3	1	10	7	10	10	17	14	20	17	23	20
<i>Festuca octoflora</i>	13	6	17	28	17	83	20	141	23	217	27	250
<i>Leucocrinum montanum</i>	7	2	7	2	7	2	13	4	20	7	20	10
<i>Pentstemon secundiflorus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hedeoma hispida</i>	0	0	0	0	0	0	3	1	3	3	3	9
<i>Astragalus tridactylus</i>	3	1	3	1	3	1	3	1	7	5	10	15
<i>Specularia leptocarpa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aragallus albiflorus</i>	0	0	0	0	0	0	0	0	0	0	3	1
<i>Cogswellia orientalis</i>	3	3	7	7	7	9	7	10	10	12	13	16
<i>Pentstemon angustifolius</i>	3	4	7	5	7	11	17	17	20	19	20	20
<i>Tragopogon pratensis</i>	3	1	3	1	7	2	10	3	17	5	23	7
<i>Collomia micrantha</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Malacothrix sonchoides</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lygodesmia juncea</i>	17	12	17	15	20	29	30	58	40	94	40	115
<i>Myosurus minimus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chenopodium album</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paronychia jamesii</i>	0	0	0	0	0	0	3	1	3	1	3	1
<i>Comandra pallida</i>	3	2	3	2	3	8	3	9	3	14	3	15
<i>Thelesperma gracile</i>	0	0	3	2	3	2	3	2	7	10	7	12

ANALYSIS OF THE DATA

In attempting to decide which of the six sizes of quadrats was the best to use, four methods of analysis were employed. The first of these was to compare the total number of species found in each size. The second was to analyze the data on abundance and the third and fourth were frequency methods.

TOTAL NUMBER OF SPECIES

The total number of species found in the quadrats in the deferred and rotation pasture ranged from 56 in the smallest size to 81 in the largest size. In the continuously grazed pasture the number of species

ranged from 58 to 80. The total number of species that have been found in the areas of both pastures is 109, and only 22 species are not recorded from any of the quadrats. Usually the individuals of these latter species are very scarce and often decidedly local in distribution. Graphs showing these variations are presented in Figure 2.

The curves representing the total number of species in the quadrats are very similar for the two pastures. Both of them show a decided tendency to flatten between the 1 and 2 square meter sizes. This flattening continues as the size of the quadrat increases. Braun-Blanquet (1) shows that in analyzing vegetation the size of the minimum area for quadrats should be where the curve begins to become horizontal.

The graph, then, indicates that the minimum size of the quadrat should not be smaller than 1 square meter, that 2 square meters is

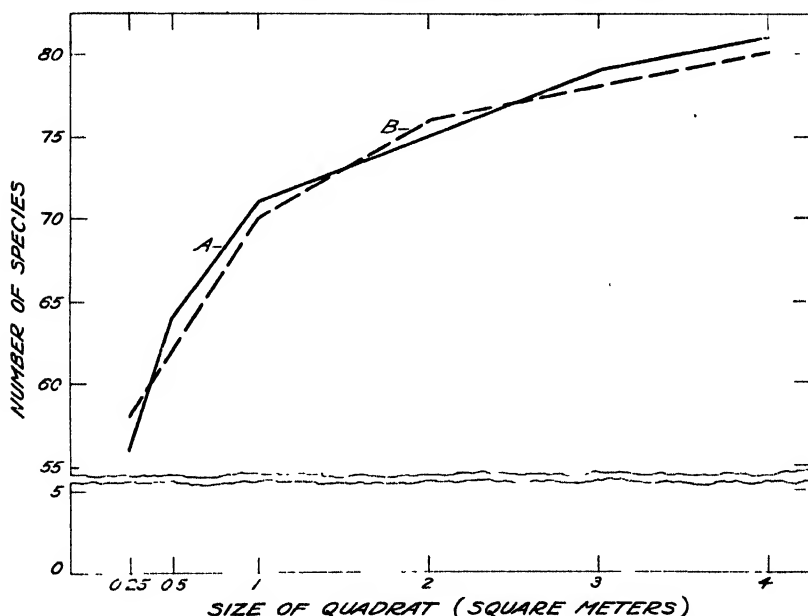


FIGURE 2.—Number of species in a total of 30 quadrats according to the size of the quadrat. A, Continuous line, represents the number of species in the deferred and rotation pasture, B, broken line, the number in the continuously grazed pasture

somewhat better, and that the benefits to be gained by using still larger sizes hardly warrant the extra time that would be required. The few additional species found in the quadrats above the 2 square meter size are so sparsely represented that they appear to be of very minor importance from the plant sociological standpoint and of no importance in range management.

ABUNDANCE

The total number of stalks of all species except sod formers or bunch grasses was secured for each size of quadrat for each pasture. Ratios were then obtained between the same size quadrats in the two pastures. In this way six comparative ratios were secured as shown in Table 3.

TABLE 3.—Total number of stalks of all species in each pasture, 30 quadrats of each size being represented

Pasture	0.25 square meter	0.5 square meter	1 square meter	2 square meters	3 square meters	4 square meters
Deferred and rotation.....	5,397	10,452	20,812	35,388	53,597	69,009
Continuous.....	3,287	6,583	13,386	24,679	38,157	50,245
Ratio.....	1.642	1.588	1.556	1.434	1.403	1.374

The average of the six ratios given in Table 3 is 1.4995 ± 0.01974 . Since this average is between those of the 1 and 2 square meter sizes it indicates that, of the sizes employed, the most desirable quadrat to use in comparing the vegetation in the two pastures lies between 1 and 2 square meters. The average ratios in each of the three separate groups of desirable, undesirable, and immaterial species also point to a size between the 1 and 2 square meter size.

FREQUENCY

The percentages of frequency for each species in Tables 1 and 2 were secured by dividing the number of quadrats in which the species was found by the total number of quadrats. For example, if a species occurred in 15 quadrats in one pasture the percentage of frequency would be secured by dividing 15 by 30 (the total number of quadrats in each pasture), giving 50 per cent.

In order to find out whether the data on frequency might indicate the use of one size of quadrat in preference to other sizes, average weighted ratios were secured (Burgess, 2, p. 16-40). For each species and for each size of quadrat the number of quadrats in which the species occurred was divided by the number of quadrats in which it occurred in the next larger size. This ratio expresses the relationship between the frequency in the two sizes of quadrats. This ratio was then weighted by multiplying it by the number of quadrats in which the species occurred in the larger of the two sizes. For example, in the continuously grazed pasture *Agropyron smithii* occurs in 30 quadrats in each of the sizes of quadrats. Therefore, 30 (in 0.25 square meter size) divided by 30 (in 0.5 square meter size) equals 1, which is the ratio of relationship. In order to weight this ratio it was multiplied by 30 (the number of quadrats in which it occurred in 0.5 square meter size), giving 30. In the case of *Schedonnardus* it is 9 (in 0.25 square meter size) divided by 13 (in 0.5 square meter size) which equals 0.69, and this ratio multiplied by 13 (in 0.5 square meter size) equals 8.97. This was done for each species in each size of quadrat.

The weighted ratios in each size of quadrat were added. The number of quadrats in which each species occurred in the next larger size of quadrat was also added. Then the former was divided by the latter to give the weighted average ratio expressing relationship of frequency of each size of quadrat to the next larger size. This method of computation may be expressed in the following formula

$$R = \frac{\sum fx}{\sum f},$$

where

R = weighted average ratio.

Σ = summation.

f = number of quadrats in which each species occurred in the larger of the two quadrats.

χ = the ratio of relationship of each species of a certain size in which each species occurred divided by the number of quadrats of the next larger size in which it occurred.

The results of this method of computation are given in Table 4.

TABLE 4.—Weighted average ratios of relationship in frequency of each size of quadrat to the next larger size

Pasture	0.25 square meter	0.5 square meter	1 square meter	2 square meters	3 square meters	Average
Deferred and rotation.	0.767	0.799	0.839	0.882	0.921	0.842 ± 0.01545
Continuous.	.790	.787	.796	.837	.935	.829 ± 0.01608

Table 4 shows that the average ratios are 0.842 ± 0.01545 and 0.829 ± 0.01608 . Since these averages are between the 1 and 2 square meter sizes the conclusion may be drawn that the size to use in comparing the vegetation should not be smaller than 1 square meter. If smaller sizes were used, then it appears that the area would not be a fair representation of the vegetation. In order for a species to be "constant" it appears that it should be present in 84 per cent or more of the quadrats in the deferred and rotation pasture and 82 per cent or more of those in the other pasture. This also points to the use of quadrats not smaller than 1 or 2 square meters.

Much work has been done in Europe on the minimum area to use in making quadrat studies in analyzing the vegetation. The term "minimum area" has been defined as the smallest area upon which the association reaches its definitive number of constants. Nichols (8, p. 134) states:

In areas of progressively smaller size than this minimum area, the number of constants rapidly diminishes; in areas of larger size, up to a certain point, it remains constant.

The minimum areas reported by Du Rietz (3, p. 423-427) usually lie between 1 and 4 square meters when a frequency of 90 per cent or more is used for the determination of constants.

The constants in the deferred and rotation pasture, using a frequency of 84 per cent as the lower limit for constancy and a minimum area of 2 square meters, are *Agropyron smithii*, *Schedonnardus paniculatus*, *Sophora sericea*, *Gaura coccinea*, and *Musineon divaricatum*. The species in the continuously grazed pasture having a frequency above 82 per cent are *Agropyron*, *Gaura*, *Musineon*, *Malvastrum coccineum*, *Sophia pinnata*, and *Psoralea tenuiflora*. The last appears to be a good indicator of overgrazing in this area.

In larger sizes of quadrats the constants increase in number, and in smaller sizes they decrease. Since the number of constants does not reach a constant number in the sizes of quadrats used for minimum frequencies between 60 and 90 per cent it might be that all of these sizes are too small to use in determining the structure of the vegetation. But for the purpose of determining the effects of different systems of grazing upon the vegetation in the two pastures it appears that the 1 or 2 square meter size is large enough.

RAUNKIAER'S LAW OF FREQUENCY

Since Kenoyer (7) has given an excellent presentation of this law it is unnecessary to describe it in detail. Raunkiaer has formulated five frequency classes. Species with frequencies between 1 and 20 per cent are in class A, 21 to 40 in class B, 41 to 60 in class C, 61 to 80 in class D, and 81 to 100 in class E. The size and number of the quadrats influence the number of species that occur in each class. Raunkiaer and Kenoyer used 25 quadrats; usually 0.1 square meter in area, for each type of vegetation. Gleason (4) prefers 100 quadrats ranging in size from 1 square meter for dense closed vegetation to 4 square meters for open or irregular communities. Both Kenoyer (7) and Gleason (5) state that the highest number of species is found in class A, that the number of species usually decreases in each class except E, which has more than class D. The curve expressing number of species in each class of frequency has two peaks, a high one in class A and a low one in class E. Gleason (5, p. 407) says that the frequency of a species is shown only when the quadrats are of such size that the resulting percentages of frequency cover the whole range of classes from A to E, and when of this size the double peak in classes A and E is often apparent. He states:

Any increase in size tends to move the index of the various species into progressively lower classes and may completely remove the peak in class V [E]. Any increase in size tends to move them into progressively higher classes and thereby to accentuate the peak in class V, while the greater total area covered continues to add more rare species to Group I [A].

TABLE 5.—Percentage of species in each class of frequency and total number of species for each size of quadrat in each pasture

DEFERRED AND ROTATION PASTURE						
Size of quadrat, sq. meter	Class A, 1 to 20 per cent	Class B, 21 to 40 per cent	Class C, 41 to 60 per cent	Class D, 61 to 80 per cent	Class E, 81 to 100 per cent	Total number of species
0.25	61	21	9	5	4	56
.5	61	17	11	6	5	64
1.0	55	20	11	7	7	71
2.0	53	16	11	12	8	75
3.0	51	14	14	14	7	79
4.0	49	10	19	11	11	81

CONTINUOUS PASTURE						
Size of quadrat, sq. meter	Class A, 1 to 20 per cent	Class B, 21 to 40 per cent	Class C, 41 to 60 per cent	Class D, 61 to 80 per cent	Class E, 81 to 100 per cent	Total number of species
0.25	74	14	7	2	3	58
.5	63	19	10	5	3	62
1.0	60	19	11	6	4	70
2.0	50	23	12	7	8	76
3.0	44	27	13	6	10	78
4.0	40	26	14	10	10	80

It appeared from these statements that a classification into frequency classes of the species in the range pastures under study might indicate the most suitable size to use. Therefore such a classification was made. The results are shown in Table 5. Analysis of this table shows that for each size of quadrat in both pastures the percentage of the total number of species is always greatest in class A and that the percentage in this class declines as the area becomes larger. In the deferred and rotation pasture the percentage of species usually becomes progressively lower as the frequency becomes higher and double peaks do not appear except in the 4 meter size, where the

second, smaller peak is in class C. The curves of all the sizes except the 4 meter size decline abruptly from class A to class B and then show a gradual tendency to become horizontal.

In the continuously grazed pasture, however, double peaks, a high one in class A and a very low one in class E, are evident in the 0.25, 2, and 3 square meter sizes. The other two sizes do not show the double peak. The curves differ from those of the other pasture in showing a less abrupt decline between the A and B classes and a slower tendency to become horizontal.

The analysis of the data, according to Raunkiaer's law, does not appear to indicate that there is any pronounced advantage in using any particular size of quadrat between 0.25 and 4 square meters. All of the sizes of quadrats give percentages in each class of frequency. The second peak, in class E, is not present in the data from the deferred and rotation pasture, and it appears in the 0.25, 2 and 3 square meter sizes in the other pasture. Perhaps still larger sizes of quadrats would have yielded a more pronounced tendency to produce peaks in class E but the extra expense entailed in using larger sizes would hardly be warranted.

SUMMARY AND CONCLUSIONS

In order to determine the most suitable size of quadrat to use in studying differences in the abundance and frequency of species in adjacent pastures grazed by two different methods, data were secured on six sizes of quadrats, ranging from 0.25 to 4 square meters. Thirty quadrats, distributed fairly uniformly and at random, were used for each size of quadrat in each pasture.

The first three methods that were used to analyze the data indicated that the size of the quadrat should not be smaller than 1 or 2 square meters. Curves showing the total number of species found in the 30 quadrats for each size of quadrat showed a decided flattening at the 1 square meter size. This continued in the 2 square meter size. The graph (fig. 2), then, indicates that the size of the quadrat should not be smaller than 1 square meter, that 2 square meters is somewhat better, but that the use of larger quadrats does not warrant the extra expense involved.

The second method of analyzing the data was to secure the ratios of the total number of stalks of plants in each size quadrat between the two pastures. Since the average of these six ratios was between those of the 1 and 2 square meter sizes it indicates that, of the sizes used, either one of these would be satisfactory.

The third method was to secure weighted average ratios of relationship in frequency of each size of quadrat to the next larger size for both pastures. Since the averages were again between those of the 1 and 2 square meter size it may be concluded that the quadrat should not comprise an area less than 1 square meter.

The fourth method, by which the data were analyzed according to Raunkiaer's law of frequency, failed to indicate any advantage in using any particular size of quadrat.

Because of the evidence secured by the first three methods and because the use of larger areas would not yield results commensurate with the additional expense, it appears that the most suitable size of quadrat to use in this type of vegetation is 2 square meters lying adjacent to each other.

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EFFECT OF CATTLE GRAZING ON VEGETATION OF A VIRGIN FOREST IN NORTHWESTERN PENNSYLVANIA¹

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INTRODUCTION

The grazing studies carried out in forest stands usually have had as their object the determination of the effect of grazing animals on the more important tree species. Little if any attention has been paid to the effect of grazing animals on forest density and composition as a whole. This is particularly true of the studies made in forests in the eastern part of the United States.

The primary object of the present study was to determine the effect of grazing upon the composition and density of vegetation in a virgin forest. In addition, an attempt was made to learn the nature and extent of the injuries caused to various species of trees by the grazing animals. Observations were made on the effect of grazing animals upon certain soil conditions.

DESCRIPTION OF THE AREAS STUDIED

The study here reported was made during the summer of 1929 in a hemlock-beech stand (more specifically, a hemlock, beech, white-pine, and red-maple stand) at Heart's Content, Warren County, in northwestern Pennsylvania. For a detailed description of this stand the reader is referred to an earlier paper by the writer (1).³ The conditions within this stand are essentially those of a virgin forest; only a relatively small amount of cutting has been done.

A portion of the area, about 25 acres, has been grazed and is inclosed by a barbed-wire fence. The adjacent forested area has not been grazed. There is every reason to believe that prior to grazing both areas supported the same kind of vegetation; relief and dominant trees on the two areas are essentially similar at present. Figure 1 shows the general conditions existing on the two areas. Adjacent to the grazed area, on the northwest side, there is a cleared field of about 5 acres which supports a grass sod. A small stream enters the grazed area along the south side and flows southward through the ungrazed area.

HISTORY OF THE GRAZED AREA

Grazing has been carried on more or less continuously for at least 20 years. It was reported by a local resident that in 1909, 5 cows were being pastured within the stand. For a period of 12 years prior

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² The writer is indebted to H. P. Clark, formerly field assistant, Allegheny Forest Experiment Station, for field assistance in gathering the data which form the basis of this report. A acknowledgment is also made to Dr. J. F. Kelly, professor of botany, Pennsylvania State College, for aid in identifying certain grasses.

Reference is made by number (italic) to Literature Cited, p. 570.

to 1928, 12 cows grazed on the area; during 1929, only 2. For a period of about 2 years, some time between 1909 and 1928, no stock were on the area. Cattle are the only animals which have been grazed, and they used the area only as night pasture from about June to October each year. In addition to the 25 acres of forest land, the stock had access to the adjacent 5 acres of grassland. Utilization of the forage within the forested area appears to have been moderately close.

METHODS

Using the barbed-wire fence which separated the grazed from the ungrazed area as a base line, strips were run out in opposite direc-

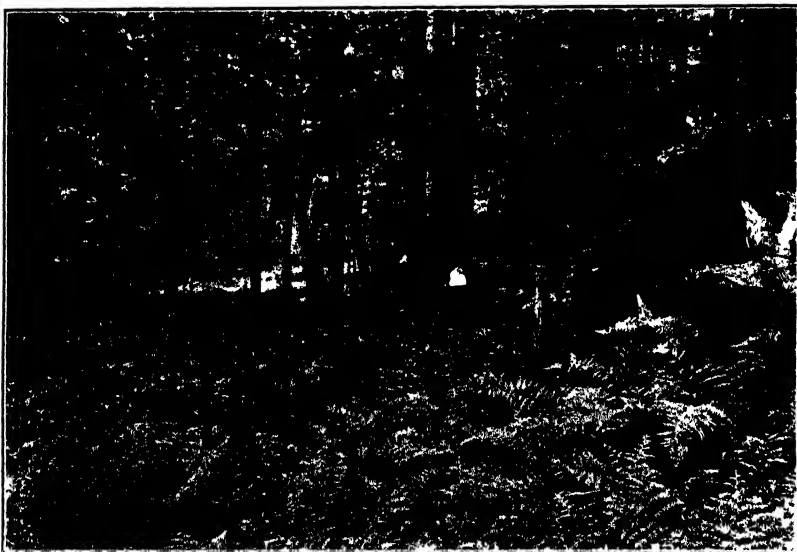


FIGURE 1.—A barbed-wire fence separates the ungrazed area (right) from the grazed area (left). Note the absence of young tree growth on the grazed side

tions into the two areas. The strips were from 100 to 200 feet apart and were established with a hand compass and 50-foot steel tape. The length of the strips varied from 200 to 500 feet; at 50-foot intervals along each strip a square quadrat of 1 milacre (6.6 by 6.6 feet, or 0.001 acre) was established. No quadrats were established within 50 feet of the fence, nor in the cleared field adjacent to the forest. On each quadrat the following data were taken:

(1) Number of individuals of each tree species in the following size classes:

Size class	Height in feet
1.....	0 to 1.0
2.....	1.1 to 2.5
3.....	2.6 to 6.0
4.....	6.1 to 15.0
5.....	15.1 to 25.0

Trees above 25 feet in height were not recorded since they were not believed to be as subject to the grazing influence as the younger trees;

forest growth above 25 feet in height appeared to be essentially the same on both the grazed and ungrazed areas.

(2) List of shrubby and herbaceous plants.

(3) Degree of cover in each of the above five size classes. Five degrees of cover were recognized:

Degree of cover	Per cent cover
1	0 to 20
2	20.1 to 40
3	40.1 to 60
4	60.1 to 80
5	80.1 to 100

(4) Diameter at breast height of all trees 0.6 inch and over.

In addition to the above data, notes were taken as to the injuries suffered by the various tree species due to grazing; the general condition of the surface soil relative to structure and consistency; the depth of the organic layers and general state of their decomposition.

The nomenclature here adopted is that of Sudworth (6) for the trees, and that of Gray's Manual (3) for the shrubs and herbs.

EFFECT OF GRAZING ON TREE SPECIES

INJURIES

The direct injuries sustained by mature trees as a result of grazing are chiefly in the roots. The extent of such injury is dependent, to a large degree, on the depth of rooting of the particular species and on the class of stock grazed. Shallow-rooted species such as beech (*Fagus grandifolia*) and hemlock (*Tsuga canadensis*) may be severely injured by heavy animals, such as cattle. The root damage at Heart's Content was most apparent in the case of beech, since root-suckers usually appeared from the vicinity of each wound and thus marked the injuries. In hemlock, the damage was not as apparent, since this species does not produce root suckers; however, there is no reason to believe that the damage to hemlock was any less severe than to beech. Besides occasionally killing the smaller roots, the injuries also open the way for fungus infection.

Browsing of young tree growth is selective and seems to be largely governed by the palatability of the various species. As a rule, damage to hardwoods is greater than damage to conifers. This fact was clearly brought out in the present study. The reduction in number of individuals per acre in size classes 2, 3, 4, and 5 which may be attributed to grazing is as follows for the three most common species: Hemlock 61 per cent, red maple (*Acer rubrum*) 81 per cent, and beech 85 per cent. That coniferous trees are not immune to browsing damage is further shown by the fact that on the grazed area about 35 per cent of the quadrats showed damage to the hemlock. A group of young hemlock trees which have been severely browsed is shown in Figure 2. Ninman and Thompson (2) in studying a grazed area in Wisconsin, found that 26.8 per cent of the white pine (*Pinus strobus*) under 20 years of age had been injured by cattle. Probably hemlock is less susceptible to browsing injury than some other coniferous species because of the relatively tough, flexible nature of its leader and branches.

The height of the young trees is also a factor in determining the extent and severity of damage by browsing and rubbing. In hemlock most of the browsing was confined to trees below 5 feet in height. Stickel and Hawley (5) state that in plantations, trees 3 feet and over in height are safe under carefully controlled grazing. Ninman and Thompson (2) found that injuries to branches of white pine were most common on trees between 3 and 7 feet in height. Sampson (4) states that aspen (*Populus tremuloides*) reproduction is no longer in danger of serious damage when the terminal shoot and some of the lateral branches have attained a height beyond which stock generally

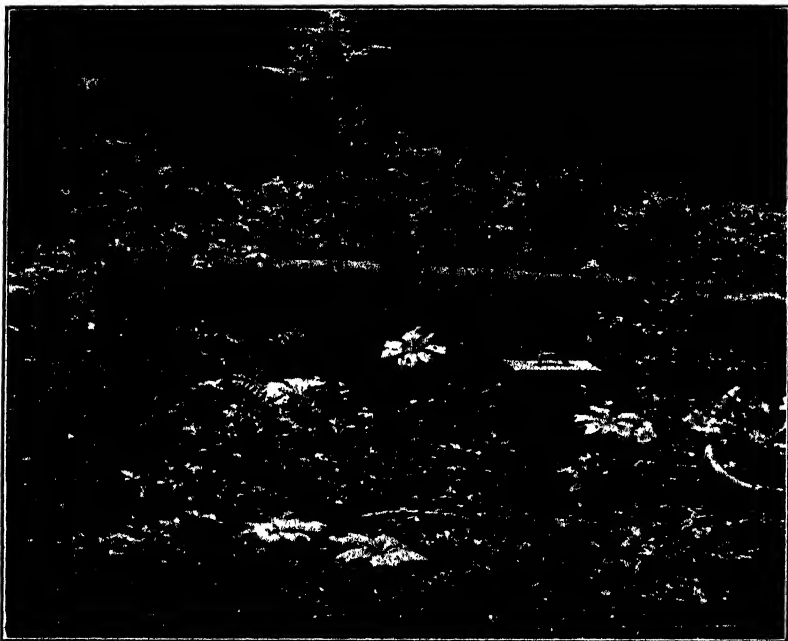


FIGURE 2.—Young hemlock 2 to 4 feet in height severely browsed by cattle

browse. He found that in the case of cattle browsing the greatest damage was done to trees under 2½ feet in height.

Hardwoods appear to suffer most severely when they are small, between 2 and 12 inches in height, and damage continues until they are 7 to 8 feet in height. Hardwoods in general are more susceptible to browsing in both smaller and larger sizes than hemlock. Damage due to rubbing by the animals is most severe when the trees are 2 to 4 inches d. b. h. (diameter at breast height), and is practically negligible in trees over 6 inches d. b. h. A few instances were noted where the stock had actually pulled hemlock trees 12 inches high out of the ground. Damage due to pulling seedlings out is most apt to occur with shallow-rooted species, or with species on shallow or excessively moist soils.

In comparison with the number of the same species dead on the ungrazed area, the dead hemlock on the grazed area was about 113 per cent and beech 134 per cent. The increased number of dead trees

of these two species on the grazed area probably is due to the influence of the grazing animals. The reason for the relatively large number of dead red maple trees on the ungrazed area and their absence on the grazed area is not apparent. Comparative numbers of dead trees found are not considered as reliable an indication of the extent of grazing damage as are comparative numbers of live trees. Because of the trampling by animals, dead trees undoubtedly disappear more quickly on grazed areas than on areas not subject to grazing.

COMPOSITION

As shown in Table 1, the composition on the ungrazed and grazed areas is practically the same so far as number of species is concerned. In the five size classes studied there are 12 species on the ungrazed area and 15 on the grazed area. Yellow birch (*Betula lutea*), hawthorn (*Crataegus* sp.), black cherry (*Prunus serotina*), and large-leaved holly (*Ilex montana*) are present on the grazed area but absent on the ungrazed area. Sugar maple (*Acer saccharum*) is present on the ungrazed area but absent on the grazed area.

TABLE 1.—Abundance and frequency of tree species on the ungrazed and grazed areas

UNGRAZED AREA *

Species	Abundance (average number per acre)					Total	Frequency index ^b (per cent)					Dead (number per acre)		
	Forest layer—						Forest layer—							
	1	2	3	4	5		1	2	3	4	5			
<i>Pinus strobus</i>	116					116	5							
<i>Tsuga canadensis</i>	4,533	967	567	450	50	6,567	70	25	25	25		167		
<i>Betula lenta</i>				17	33	50					5			
<i>Fagus grandifolia</i>	606	217	367	633	250	2,133	15	15	20	40	15	83		
<i>Castanea dentata</i>			33	17		50			5			17		
<i>Quercus borealis maxima</i>	33			17		50	5							
<i>Magnolia acuminata</i>	67	33	17	17		134	5							
<i>Hamamelis virginiana</i>	83					83								
<i>Amelanchier canadensis</i>		17		17		34								
<i>Prunus pennsylvanica</i>	33					33	5							
<i>Acer saccharum</i>	17					17								
<i>Acer rubrum</i>	2,550	283	67	200	17	3,117	60	15	5	15		83		

GRAZED AREA *

<i>Pinus strobus</i>	95					95	10					
<i>Tsuga canadensis</i>	5,603	381	174	190	47	6,395	75	15	10	10	5	190
<i>Betula lenta</i>	80	16				96	5					
<i>Betula lutea</i>	16					16						
<i>Fagus grandifolia</i>	8,381	111		95	16	8,603	50	5		5		111
<i>Castanea dentata</i>	32					32	5					16
<i>Quercus borealis maxima</i>	47					47	5					
<i>Magnolia acuminata</i>	63					63	5					
<i>Hamamelis virginiana</i>	16	111	47			174						
<i>Amelanchier canadensis</i>	32					32	5					
<i>Crataegus</i> sp.....	47					47						
<i>Prunus serotina</i>	63					63	5					
<i>Prunus pennsylvanica</i>	32					32	5					
<i>Ilex montana</i>		16				16						
<i>Acer rubrum</i>	4,000	32		63	16	4,111	80			5		

* Based on 60-milacre plots.

* Expressed in class intervals of 5.

* Based on 63-milacre plots.

The abundance and frequency values of the three most common species, hemlock, beech, and red maple, are decidedly different on the two areas. In each case the abundance of these species in size class 1 was much higher on the grazed than on the ungrazed area. The

increase in numbers was greatest in the case of beech. On the grazed area the number of beech trees in size class 1 is about 1,258 per cent of the number in the same size class on the ungrazed area. The large amount of beech in size class 1 on the grazed area is practically all of root-sucker origin and most of it is only 1 or 2 inches high. It has been repeatedly browsed off by cattle. Even if protection were afforded it seems doubtful that the suckers would grow into good forest trees. The increased numbers of hemlock and red maple trees in size class 1 on the grazed area may be due to more favorable seed-bed conditions and increased light.

In size classes 2, 3, 4, and 5 on the grazed area the abundance and frequency values of hemlock, beech, and red maple are decidedly lower than on the ungrazed area. (Table 1.) Red maple, particularly, appears to be almost entirely eliminated on the grazed area. It seems clear that continued grazing prevents full reproduction of the tree species, and in time will result in decreased density of the main crown canopy. Likewise it appears that the resultant forest stand will contain a relatively higher percentage of hemlock than the original forest.

BASAL AREA

Table 2 shows the basal areas of the young trees on the grazed and ungrazed plots. The basal-area values given represent trees 1 inch and over in diameter at breast height in size classes 3, 4, and 5. The basal area on the grazed side is about 41 per cent of that on the ungrazed side.

TABLE 2.—Basal area of young trees on ungrazed and grazed areas

Species	Basal area on un- grazed area ^a	Basal area on grazed area ^a	Species	Basal area on un- grazed area ^a	Basal area on grazed area ^a
	Sq. ft. per acre	Sq. ft. per acre		Sq. ft. per acre	Sq. ft. per acre
<i>Tsuga canadensis</i>	1 86	4. 11	<i>Hamamelis virginiana</i> ..	. 10	---
<i>Betula lenta</i>	73	---	<i>Acer rubrum</i>	3 86	73
<i>Fagus grandifolia</i>	12 51	3 00	Total.....	19 26	7 84
<i>Quercus borealis maxima</i> 10	---			
<i>Magnolia acuminata</i>	10	---			

^a Represented by trees 1 inch or more d. b. h., occurring in size classes 3, 4, and 5.

EFFECT OF GRAZING ON LESSER VEGETATION

COMPOSITION

Table 3 lists all the shrubby and herbaceous species found on the two areas, together with their frequency values in terms of percentage. Of the total of 70 species recorded, 27 are common to both areas. Nine species were found on the ungrazed area only and 34 species were found only on the grazed area. The following species of relatively high frequency are confined largely or wholly to the ungrazed area: *Lycopodium lucidulum*, *Coptis trifolia*, and *Viburnum alnifolium*. Apparently the conditions resulting from the presence of grazing animals inhibit the development of these species on the grazed area.

TABLE 3.—Frequency of shrubby and herbaceous species on the ungrazed and grazed areas

[Based on 60 ungrazed plots, 63 grazed]

Scientific name	Common name	Frequency (per cent)	
		Ungrazed area	Grazed area
<i>Aspidium noveboracense</i>	New York fern		8
<i>Aspidium spinulosum</i>	Common wood fern	78	88
<i>Dicksonia punctilobula</i>	Hay-scented fern		22
<i>Onclea sensibilis</i>	Sensitive fern		2
<i>Osmunda claytoniana</i>	Interrupted fern	2	(*)
<i>Osmunda cinnamomea</i>	Cinnamon fern	7	
<i>Lycopodium lucidulum</i>	Shining club moss	22	2
<i>Anthoxanthum odoratum</i>	Sweet vernal grass		3
<i>Brachelytrium erectum</i>	Bearded short-husk	2	
<i>Phleum pratense</i>	Timothy		(*)
<i>Agrostis alba</i>	White bent grass		(*)
<i>Poa sp.</i>	Meadow grass		(*)
<i>Juncus tenuis</i>	Slender rush		2
<i>Oxckesia sessilifolia</i>	Sessile-leaved bellwort	7	10
<i>Clintonia umbellulata</i>	Speckled clintonia	3	
<i>Muianthemum canadense</i>	Wild lily-of-the-valley	13	50
<i>Streptopus roseus</i>	Rosy twistedstalk	2	(*)
<i>Medeola virginiana</i>	Cucumber root	3	3
<i>Trillium erectum</i>	Wake-robin	3	3
<i>Habenaria orbiculata</i>	Roundleaf orchis	2	(*)
<i>Corallorrhiza maculata</i>	Coralroot	2	
<i>Laportea canadensis</i>	Wood nettle		2
<i>Pilea pumila</i>	Richweed	2	18
<i>Rumex obtusifolius</i>	Bitter dock		14
<i>Rumex acetosella</i>	Field sorrel	2	3
<i>Polygonum hydropiper</i>	Common smartweed	3	21
<i>Phytolacca decandra</i>	Common pokeberry		2
<i>Stellaria media</i>	Common chickweed		(*)
<i>Ranunculus acris</i>	Tall buttercup		6
<i>Coptis trifolia</i>	Gold thread	20	
<i>Codophyllum peltatum</i>	Mayapple		6
<i>Cardamine pennsylvanica</i>	Bittercress		(*)
<i>Fragaria virginiana</i>	Strawberry		3
<i>Potentilla recta</i>	Rough-fruited cinquefoil		3
<i>Potentilla canadensis</i>	Fivefinger		10
<i>Rubus occidentalis</i>	Black raspberry		(*)
<i>Rubus allegheniensis</i>	Mountain blackberry		3
<i>Rubus hispidus</i>	Swamp dewberry	2	3
<i>Rubus sp.</i>	Rubus	3	11
<i>Dalibarda repens</i>	Dalibarda	7	
<i>Trifolium pratense</i>	Red clover		3
<i>Trifolium hybridum</i>	Alsike clover		2
<i>Trifolium sp.</i>	Clover		2
<i>Oxalis acetosella</i>	Common wood sorrel		48
<i>Oxalis corniculata</i>	Lady's sorrel		2
<i>Viola papilionacea</i>	Early blue violet	18	58
<i>Viola canadensis</i>	Canada violet	2	
<i>Circaea intermedia</i>	Enchanter's nightshade	8	24
<i>Aralia nudicaulis</i>	Wild sarsaparilla	7	
<i>Osmorhiza longistylis</i>	Sweet cicely	2	(*)
<i>Cornus alternifolia</i>	Alternate-leaved dogwood	2	
<i>Monotropa uniflora</i>	Indianpipe		2
<i>Rhododendron nudiflorum</i>	Purple azalea	7	3
<i>Gaultheria procumbens</i>	Wintergreen	2	2
<i>Vaccinium pennsylvanicum</i>	Low sweet blueberry	3	(*)
<i>Trientalis americana</i>	Starflower	3	16
<i>Prunella vulgaris</i>	Heal-all		3
<i>Veronica officinalis</i>	Common speedwell		5
<i>Epipagus virginiana</i>	Beechdrops	10	14
<i>Plantago major</i>	Common plantain		2
<i>Gallium triflorum</i>	Sweet-scented bedstraw	2	3
<i>Mitobella repens</i>	Partridge berry	68	53
<i>Viburnum alnifolium</i>	Hobblebush	53	16
<i>Viburnum acerifolium</i>	Mapleleaf viburnum	2	2
<i>Lobelia inflata</i>	Indian tobacco		(*)
<i>Sambucus canadensis</i>	Common elder	10	10
<i>Sambucus racemosa</i>	Red-berried elder	3	2
<i>Aster sp.</i>	Aster		2
<i>Bidens discolor</i>	Small beggar-ticks		6
<i>Hieracium aurantiacum</i>	Devil's-paintbrush		3

* Species observed within the area but not encountered on plots.

The following species, most of which have a frequency of 10 per cent or more, are confined largely or wholly to the grazed area: *Aspidium noveboracense*, *Dicksonia punctilobula*, *Pilea pumila*, *Rumex obtusifolius*, *Polygonum hydropiper*, *Potentilla canadensis*, *Oralis acetosella*, and *Circaea intermedia*. It is interesting to note the high frequency (48 per cent) of *O. acetosella* on the grazed area and its total absence in the hemlock-beech stand on the ungrazed area. Earlier work by the writer (1) at Heart's Content showed that this species was absent in the hemlock-beech association but was very common (frequency 40 per cent) in the hemlock consociation. On the grazed area, in the hemlock-beech association, the frequency of *O. acetosella* is even higher than in the ungrazed hemlock consociation. The writer can offer no explanation for this rather striking condition. Troup (?) states that in Europe the wood sorrel is one of the best indicators of favorable light and soil conditions in a forest.

Other species which, although having low frequency values, are considered characteristic of the grazed area are: *Anthoxanthum odoratum*, *Phleum pratense*, *Agrostis alba*, *Poa* sp., *Juncus tenuis*, *Phytolacca decandra*, *Stellaria media*, *Ranunculus acris*, *Podophyllum peltatum*, *Cardamine pennsylvanica*, *Potentilla recta*, *Trifolium pratense*, *Trifolium hybridum*, *Trifolium* sp., *Prunella vulgaris*, *Plantago major*, *Lobelia inflata*, *Bidens discordea*, and *Hieracium aurantiacum*.

The number of herbaceous species on the grazed area is about 189 per cent of the number of species found on the ungrazed area. Soil conditions resulting from the presence of stock appear more favorable for the development of a rather rich herbaceous vegetation. The more favorable conditions appear to consist of increased light, better seed-bed conditions due to the trampling of the animals, and reduced competition from shrubby and young tree growth. In addition, it is probable that stock may play an important rôle in seed dissemination. The seeds of such a plant as *Bidens discordea* may be carried on the bodies of animals; seeds of *Phleum pratense*, *Trifolium pratense*, *T. hybridum* and others may be deposited with the dung. Some of the species appearing almost exclusively on the grazed area are considered to be nitrophilous, e. g., *Pilea pumila* and *Circaea intermedia*.

EFFECT OF GRAZING ON DENSITY OF VEGETATIVE COVER

Figure 3 shows graphically the effect of grazing animals on the vegetation as a whole. The degree of cover, in terms of percentage, for each of five size classes in the grazed and ungrazed areas is indicated in solid black. Shrubby and herbaceous vegetation, as well as young tree growth, were considered in arriving at the degree of cover. In the smallest size class—the vegetative layer up to 1 foot in height—the degree of cover on the grazed area is almost twice that on the ungrazed area. Moreover, no other size class on the grazed area has so high a degree of cover. This high density is due chiefly to the increase in amount of herbaceous vegetation. The size classes above the general limit of herbaceous vegetation show a different condition. The degree of cover on the grazed area is only about 58 per cent of that on the ungrazed, in size class 2; 17 per cent in size class 3; 62 per cent in size class 4; and 102 per cent in size class 5. The effect of grazing thus appears to be maximum in the vegetative layer between 2.6 and 6 feet. In the next tallest layer, the com-

ponents of which probably have been longest exposed to browsing, grazing has reduced the density of cover from 38 per cent in the ungrazed area to 24 per cent in the grazed—nearly as great an absolute reduction as in size class 3. The practical uniformity, on both sides of the pasture fence, of cover density in the 15.1 to 25 foot size class indicates that the vegetation in this class was too tall when grazing began to have suffered appreciably from it.

EFFECT OF GRAZING ON THE SITE

No direct study of the effect of grazing on the site was carried out but observations were made during the course of the other field work. Trampling by the stock appears to have given the mineral surface soil on the grazed area a rather dense structure and compact consistency. From the standpoint of forest growth, this condition may be regarded as unfavorable. On the other hand, the presence of animals on the grazed area appears to have encouraged rapid decomposition of the organic debris. Over most of the area the humus

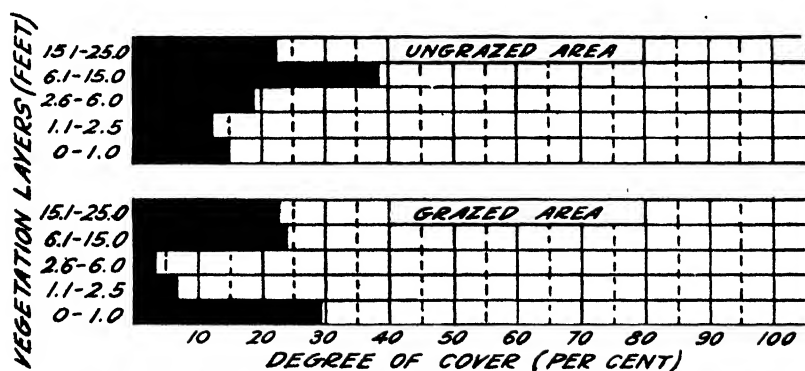


FIGURE 3.—Degree of cover in each of five size classes on the ungrazed and grazed areas

conditions appear considerably better than on the ungrazed area. Rather rapid nitrification is indicated by the fairly common occurrence of species which are generally regarded as nitrophilous. A study of the effect of grazing animals on the physical and chemical characteristics of the forest soil would be valuable.

SUMMARY

The effect of grazing on virgin forest vegetation was studied at Heart's Content, in northwestern Pennsylvania.

Direct injury to vegetation by grazing animals consists of browsing the tops, breakage by rubbing or trampling, root injuries due to trampling, and pulling small trees out of the soil. In the case of hemlock and beech a greater number of dead trees were recorded on the grazed than on the ungrazed area. However, comparative numbers of dead trees on the two areas are not considered as good an index of the severity of grazing injury as are comparative numbers of live trees.

Compared with the ungrazed area, the number of individuals of hemlock, red maple, and beech in size class 1 showed a large increase on the grazed area. In size classes 2, 3, 4, and 5, however, there was

a marked reduction in the number of individuals per acre on the grazed area. This reduction amounted to 61 per cent in the hemlock, 81 per cent in the red maple, and 85 per cent in the beech.

The basal area of trees 1 inch and over, diameter at breast height, in size classes 3, 4, and 5 on the grazed area was 7.84 square feet and on the ungrazed area was 19.26 square feet.

The total number of shrubby and herbaceous species was much greater on the grazed than on the ungrazed area. A number of species appeared exclusively on the grazed and others appeared exclusively on the ungrazed area.

The degree of cover on the grazed area in the five size classes recognized was in general notably lower than the degree of cover in the same size classes on the ungrazed area.

The surface soil on the grazed area was compacted due to the trampling by stock. Another result of the presence of the stock was the reduction in the amount of organic debris on the forest floor due to more rapid decomposition. A number of the plants on the grazed area are commonly recognized as nitrophilous.

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WINTER SOIL TEMPERATURES AND THEIR RELATION TO SUBTERRANEAN INSECT SURVIVAL¹

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INTRODUCTION

The studies on winter soil temperatures discussed in this paper are a development of work done at the University of Minnesota on wireworms (*Elateridae*) during the winter of 1927-28. The data obtained are, however, of wide application, affecting as they do every form of insect life which is passed in the soil in any stage, be it egg, larva, pupa, or adult. Not only entomologists but also agronomists and engineers are interested in heat conduction through soil, though the interests of the latter are in depths much below those studied here. Nevertheless, a similar technic to that here described could readily be used in following temperature waves downward to any required depth.

It has, of course, long been known that a snow blanket insulates the soil against subzero air temperatures. But, numerous as have been the workers on soil temperatures, there can be found but few instances where exact measurements have been made of the degree of protection which snow affords.

Swezey (18),³ Bouyoucos (1, 2), and McColloch and Hayes (9) have, among others, made extensive soil-temperature studies, but from different angles and with varied technic. The work of Swezey is valuable because it represents the records of a long term of years and at a variety of depths. Bouyoucos studied the influence of the various intrinsic factors affecting soil temperature to a depth of 18 inches under controlled and natural conditions, and the relationship between the soil temperature and the different meteorological elements. McColloch and Hayes, like the writer, were interested in soil temperature from the standpoint of the insect survival, but their technic was altogether different from that herein described. They were interested primarily in the effect of soil temperatures on the migration of white grubs in the larval and adult stages, whereas this paper deals with the effect of subzero temperatures on insects which normally overwinter in the upper 2 feet of soil.

The records here presented cover a period of eight months, from October 1 to June 6, by which date spring conditions had been established.

¹ Received for publication Apr. 15, 1930; issued October, 1930. Published with the approval of the director as paper No. 906 of the Journal series of the Minnesota Agricultural Experiment Station. The bulk of the work on which this paper is based was done in the laboratories of the division of entomology, University of Minnesota, and a small portion at the Montana Agricultural Experiment Station.

² The writer gratefully acknowledges his indebtedness to Dr. William Robinson, Dr. R. N. Chapman, Prof. A. G. Ruggles, Dr. F. G. Holdaway, and H. E. Gray for helpful criticisms and assistance.

³ Reference is made by number (italic) to Literature Cited, p. 591.

METHODS AND APPARATUS

The thermocouple method of taking soil temperatures was used in preference to that of soil thermometers because of its simplicity and accuracy. Connell (4) describes an efficient method of taking soil temperatures, using an iron pipe and standard thermometer, but for winter work this method would be much less convenient. Sweetman used the thermocouple method at Minnesota for two years with good results, and the apparatus herein described is a modification of his set-up. Robinson (15) has described the principle of the thermocouple so very clearly that any discussion here is needless.

It was desired to obtain soil temperatures at intervals of 2 inches (5.08 cm.) from soil surface to a depth of 2 feet (60.96 cm.), in two different situations, one where the ground was allowed to retain its normal covering of snow and the other under bare earth. The buried part of the apparatus consisted of two pieces of 1 by 12 inch board 3 feet long, to which all the junctions were affixed. The object of attaching them to a solid base was to facilitate the sinking of the junctions to the required depth and to insure that they would maintain their relative positions. Each board was, in this particular instance, prepared by boring holes three-sixteenths of an inch in diameter, these holes being staggered on each side of a center line and with a 4-inch vertical distance between the holes. A constantan wire of 28 gage was led down the center of the board to the 24-inch hole where a junction was made with a copper wire of the same gage. The constantan wire was tapped by short leads to the remaining holes and at each hole a junction was made with a copper wire. The reason for having the junctions separated by a horizontal distance of about a foot was to insure that the temperature set up by one junction would not affect the reading of the next junction only 2 inches distant. The 12 copper wires had been previously shellacked and twisted into 40-foot cables and these inclosed in two lengths of $\frac{1}{8}$ -inch rubber tubing as insulation. (These cables can, of course, be of any desired length within reasonable limits, depending on the distance of the buried junctions from the recording instruments.) The copper-constantan junctions were then pushed through the holes in the board until they projected about $1\frac{1}{2}$ inches. A $\frac{3}{16}$ -inch shell vial slipped over each junction and fitted tightly into the hole. No wires were left exposed, all short leads being insulated in rubber tubing. Absorbent cotton soaked in shellac was plugged on each side of any break in the rubber insulation to prevent air circulation and to make the system as nearly waterproof as possible. All breaks in the rubber tubing were wound with rubber and friction tape to render the joints waterproof. The network of insulated wires was then fastened securely to the board with clamps. (Figs. 1 and 2.)

Holes of the required depth were dug and the boards placed therein in a vertical position. The earth removed was well puddled with water and poured slowly into the holes so that the junctions would be thoroughly embedded. After the excess water was drained off, the resulting depressions were filled to the level of the ground surface and the system left for some weeks to come to equilibrium with the surrounding soil before readings were taken.

The constantan wires from each board were soldered together and a single constantan lead was run to the switchboard.

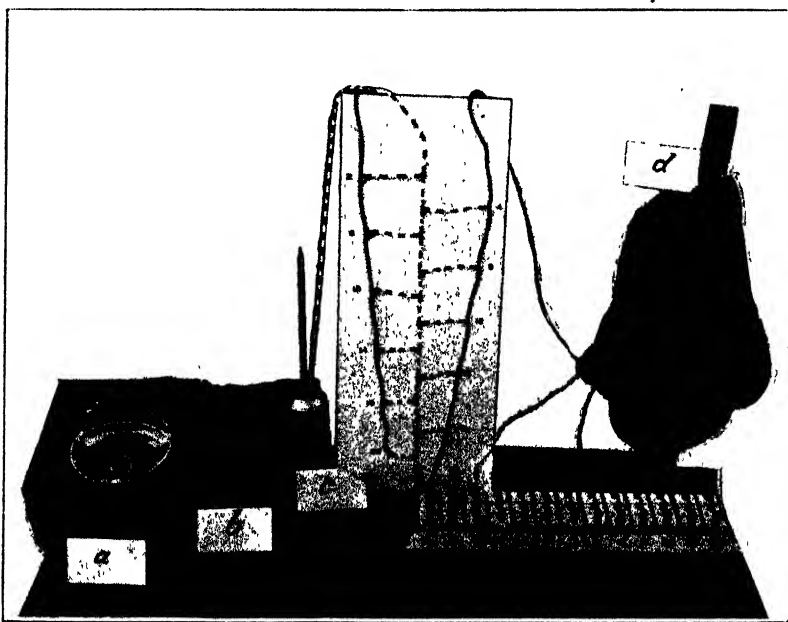


FIGURE 1 --Apparatus used for measuring soil temperatures *a*, Pyrovoltter, *b*, outside galvanometer, *c*, thermos bottle with junction at known temperature, *d*, 40 feet of wire cable connecting inside switchboard with buried junctions

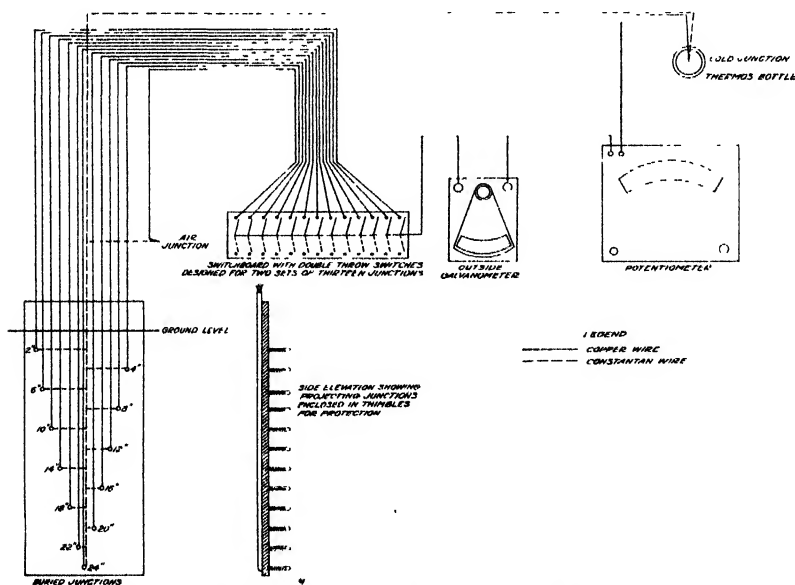


FIGURE 2.-- Diagram of system of wiring used in soil-temperature apparatus

The rest of the apparatus was erected on a table inside a heated building adjacent, so that readings could be taken at any time and under any weather conditions. The 12 leads from the snow-covered junctions were connected to the poles of a battery of single-pole double throw switches. The leads from the bare-earth junctions were connected to the other terminals. The constantan wire was led to the cold junction which was in a thermos bottle containing cracked ice and water at 0° C. registering the known temperature. The copper wire from this "known" junction was connected through a pyrovoltmeter and outside galvanometer to the common terminals of the switch-board.

The throwing in of a switch gave the reading at a single depth and in either of the two situations, depending on which way the switch was thrown. An additional junction for air temperature was also added to the system, this being fastened to the side of a post outside and in the shade.

Temperatures were taken every two days during most of the period of the study, and at about 8 o'clock in the morning. As the days lengthened in the spring, readings were taken at an earlier hour.

The mean air temperature was plotted from the records of the United States Weather Bureau, Minneapolis station.

SOIL-TEMPERATURE DATA

There are two sources of heat by which soil temperature may be affected (1) the radiant energy from the sun, and (2) internal heat conducted to the surface from below. The internal heat of the earth, in which the temperature rises 1° F. for every 100 feet of penetration into the earth, is negligible in these experiments, as only the first 2 feet of soil are being studied.

In temperate regions, daily variations of temperature cease to be felt at depths ranging from 2 to 3 feet, according to the nature and condition of the soil. Monthly variations are felt to much greater depths, and in the Temperate Zone, e. g., Paris and Brussels, annual variations do not disappear at a depth less than 75 feet (7, *p.* 302-303). Smith (17, *p.* 80) puts the limit of annual variation at 40 feet in depth, but in any case it is too deep to be of any significance in a study involving merely the surface layers. The solar heat descends in waves, the effect of which can be followed for some days at the lower depths. The effect of a few warm days before a sudden cold spell is still in evidence after some days of reduced temperature. This lag is clearly demonstrated in Figure 3, where two days of warm weather between October 9 and 11 caused a corresponding rise immediately at the 2-inch level but did not affect the 8-inch level until October 13, and the 16-inch level until October 16. The effect of sudden rapid variations in temperature is not felt at depths much beyond 1 foot, only spells of warmth or coolness lasting for more than a day being able to affect the greater depths. The temperature of the upper layers follows closely the atmospheric changes, but the farther one penetrates the smoother is the curve obtained, and at 2 feet daily fluctuations are very small. The weekly variations at 2 feet are, of course, greater, and the seasonal variation is still more pronounced at that depth. Thus we find, in the fall and spring of the year, when hot days and cool nights are the rule, that the shallower depths follow,

with a decreasing correspondence, the fluctuations of the air temperature, while the lower depths show rather a correspondence with the

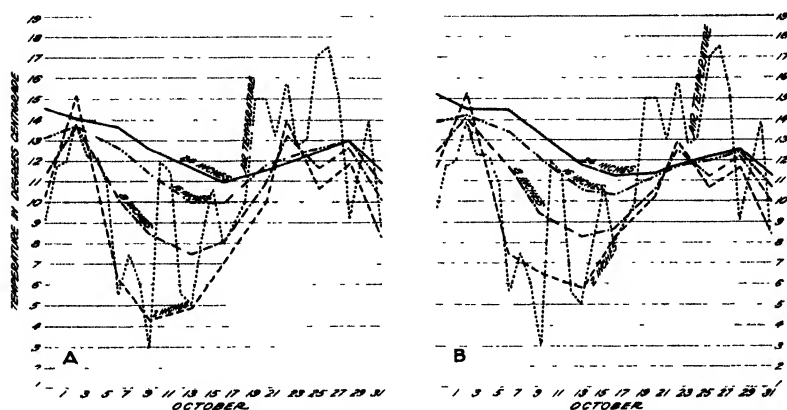


FIGURE 3. Air temperatures and soil temperatures at different depths in bare (A) and sod-covered (B) ground during October

means of increasingly longer intervals. (Figs. 3, 4, 5, 6.) Under these conditions the charted lines for the shallower depths will cut

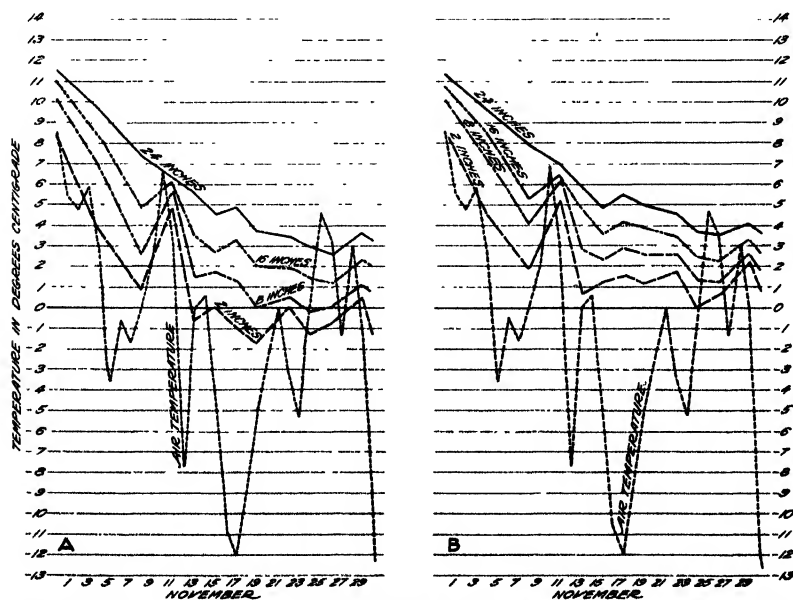


FIGURE 4.--Air temperatures and soil temperatures at different depths in bare (A) and sod-covered (B) ground during November

those of the depths more remote from the surface in several places; as witness the situations figured on October 1 and 22 (fig. 3), January

13 (fig. 7), February 7 (fig. 8), and April 16 (fig. 5), to quote only a few instances. At these points, contrary to the expectation in winter

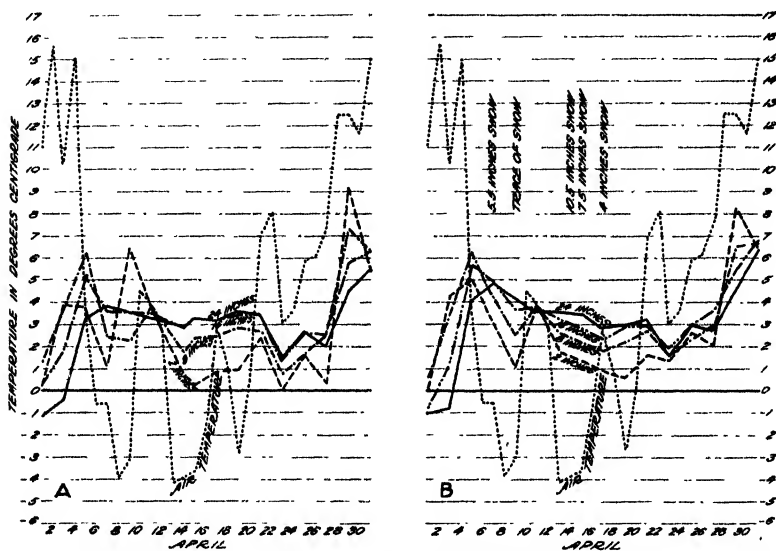


FIGURE 5 - Air temperatures and soil temperatures at different depths in bare (A) and snow-covered (B) ground during April

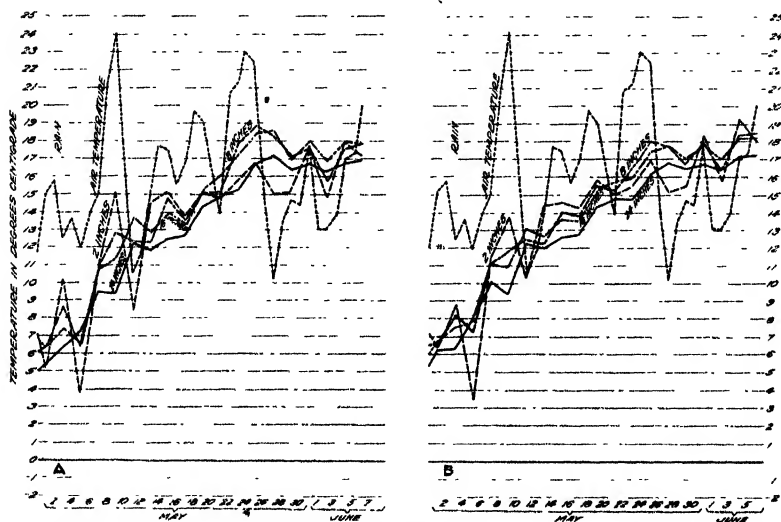


FIGURE 6 - Air temperatures and soil temperatures at different depths in bare (A) and sod-covered (B) ground during May and early June

temperatures, the 24-inch level is coldest, the 16-inch level slightly warmer and so on until the 2-inch level is reached, which shows the highest temperature.

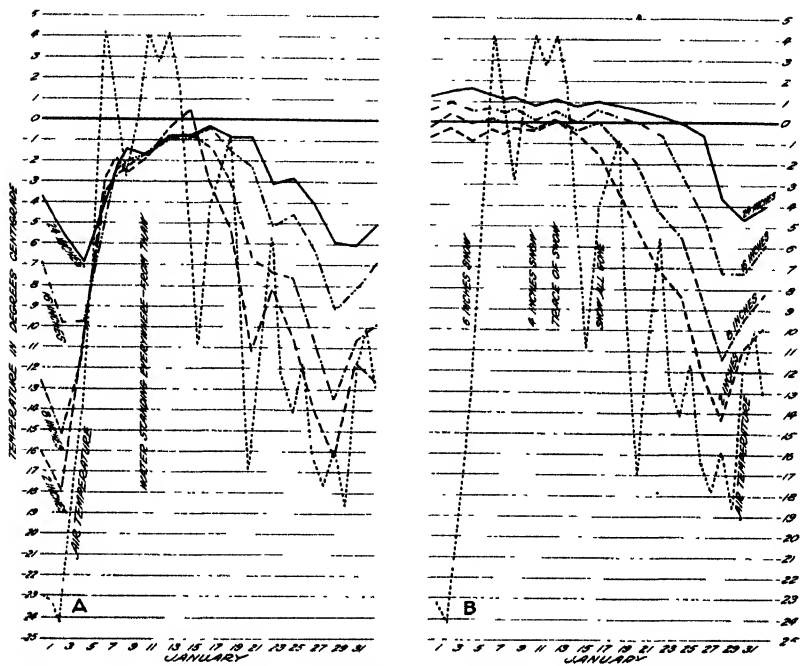


FIGURE 7—Air temperatures and soil temperatures at different depths in bare (A) and snow-covered (B) ground in January

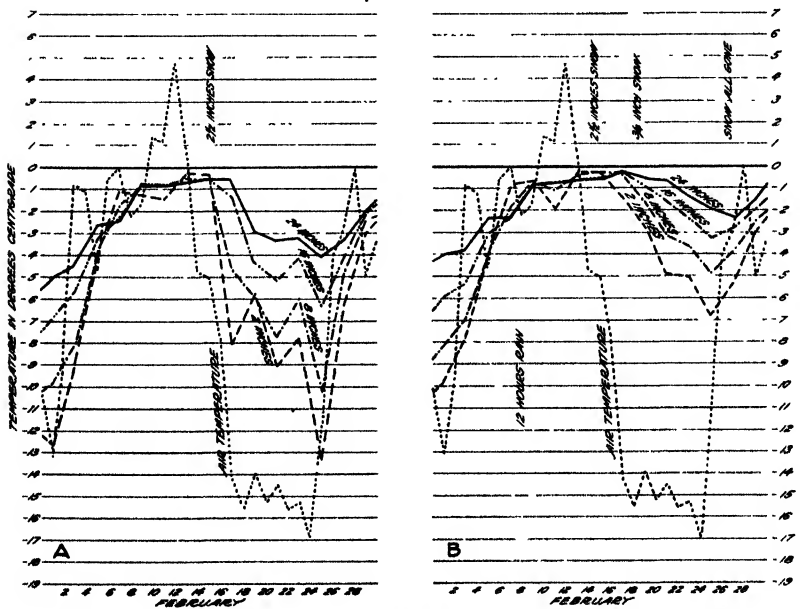


FIGURE 8—Air temperatures and soil temperatures at different depths in bare (A) and soil-covered (B) ground in February

FALL AND SPRING INVERSION

We find, in lakes, a temperature stratification with the warm and disturbed epilimnion above, the stable and cool hypolimnion below, and the shifting but definite thermocline between them. In the spring and fall the waters of the whole lake are of about uniform density and we observe the phenomenon of a complete circulation of all the water. Similarly in the soil, while the absence of circulation inhabits a true turnover in the sense in which it is found in lakes, yet there is a temperature movement which is to some extent comparable. At these times a complete reversal of temperature conditions is observed. In warm countries there are two times in the day when the direction of heat movement is changing. During the day heat is moving downward in the upper layer of the soil and during the night it is moving upward. The point where there is momentarily no movement is called by McKenzie and Williams (12) the thermostatic point. In this study, because of the time between observations, these thermostatic points are only in evidence in the fall and spring, their positions being indicated by the crossing of the temperature curves for the different levels. During the hot summer months the surface layers of the soil are much warmer than are the lower layers. During the cold months the soil at greater depths is warmer. This inversion commonly takes place in the fall and spring in temperate regions, though unusually warm spells in the winter will cause partial inversions. A complete inversion, however, only occurs when the mean air temperature is above the freezing point. Fully as great fluctuations of the mean air temperature as is shown on the dates mentioned above, occur in November (fig. 4) and during practically the whole month of December (fig. 9), but if these fluctuations occur when the mean air temperature is less than 0° C., the inversions do not take place. In several instances where a series of fluctuations culminated in a rise of the mean air temperature to almost 0° an inversion almost occurred, there being less than a degree of difference in spread between the 2-inch and 24-inch levels, and on February 6 (fig. 8) the inversion occurred when the mean air temperature reached 0° , but here the spread between the top and bottom levels was less than 1.5° .

In the spring of the year the air means fluctuate rapidly and sharply and the succeeding warm and cold waves through the soil result in a chaotic crisscrossing of the temperature lines during this period with, however, a relatively small spread. During the winter months, on the other hand, fluctuations of equal rapidity and spread do not so markedly affect the internal soil temperatures, and a greater spread is maintained between the temperatures of the upper and lower levels. In general, it would appear that variations of the air temperature which occur above 0° C. affect the internal soil temperatures to a much greater degree than do similar fluctuations below the zero line, i. e., in the absence of snow or other insulation. The reason, of course, why subzero temperature changes do not so markedly affect the internal soil temperature is that in winter the ice in the soil has first to be melted, the heat of fusion being 80.02 gm. calories. Water possessing such a high heat capacity, five times greater than that of dry soils, requires five times as much heat to raise the temperature of a unit of water to 1° as is necessary for a unit weight of soil.

As the specific heat of water is so much higher than that of any soil it therefore follows that masses of water act powerfully in the moderation of soil temperature by their mere presence. The immediate effect of a warm wave is not evident in ice-filled soils and a lag results. A rise in air temperature from -23° to -6° C., that is 17° , through five days, resulted in a corresponding rise in the 24-inch level of only 0.75° five days after the commencement of the upward movement. But an almost corresponding situation in the above-zero part of the scale, that is a rise of the mean air temperature from -3° to $+15^{\circ}$, or 18° in five days, resulted in a rise of the temperature of the 24-inch level of 5° , five days after the commencement of the upward movement. Here is a comparable rise in air temperature above and below the zero line, but the effect on the deeper soil temperatures compared to those of the upper strata is markedly different.

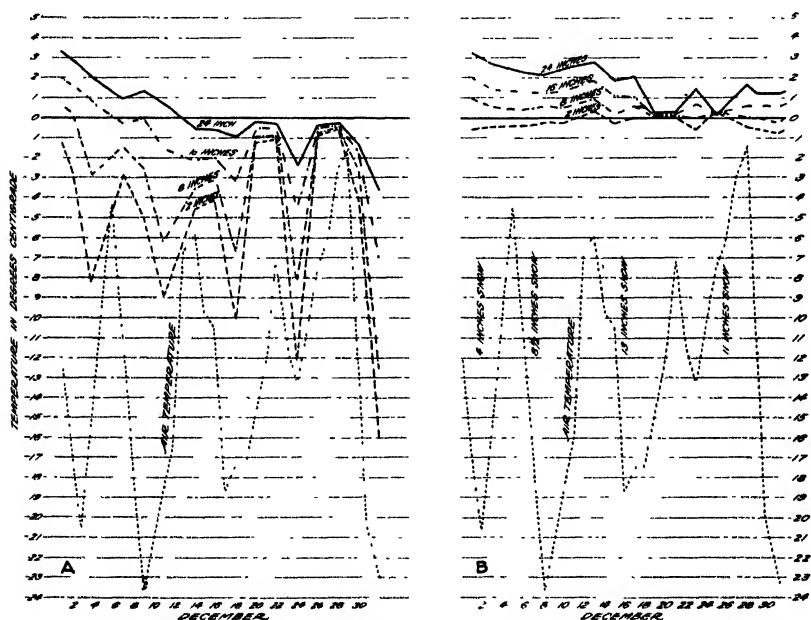


FIGURE 9.—Air temperatures and soil temperatures at different depths in bare (A) and snow-covered (B) ground in December

McKenzie-Taylor and Williams (12) show in their study of sand temperatures that the reduction in temperature range was approximately constant for a constant increase in depth. Given a known surface temperature they were able to calculate mathematically the temperature at any depth, and the observed and calculated temperatures were in remarkably close agreement. With soil, however, this agreement between the calculated and observed range was absent. Soil, unlike sand, is so lacking in uniformity of composition, and so many factors enter into the question of its heat conduction that ordinarily it is almost impossible to compute mathematically the temperature at a given depth from a known surface temperature.

Extremes of temperature, i. e., maxima and minima, especially during the winter months are more significant than monthly or even

weekly means in so far as the ability of an insect to hibernate is concerned.

EFFECT OF SNOW ON TEMPERATURE OF THE SOIL

The temperature records obtained from a situation where there is a surface insulation of snow differ, as one would anticipate, very markedly from those from bare ground subjected to the same air-temperature conditions. Bouyoucos (2) studied the effect of snow on the soil temperature for several winters, and for one month of his studies made comparisons of soil temperatures under the following conditions: (1) Bare, (2) covered with compacted snow, (3) covered with uncompacted snow, and (4) covered with a layer of vegetation and uncompacted snow. His findings are very similar to those shown here. A comparison of Figures 3 and 4 shows that for the months of October and November the temperatures in both the bare and sod-covered ground paralleled each other very closely, the slight lag in the temperature lines of the latter being due to the insulating effect of the sod covering. On December 1, 3½ inches of snow fell, and during the period from the first to the fifteenth there accumulated 15 inches of snow, which did not wholly disappear until January 12. From November 30 until January 5 the mean air temperature was never above 0° C., and for several days was below -18°, reaching a minimum of -24.2° on January 2. The temperatures recorded for the bare ground during this period closely followed the sharp fluctuations of the air temperature, the lowest temperatures recorded from the different levels being: Air, -24.2° C.; 2-inch level, -17.94°; 8-inch, -15.24°; 16-inch, -9.78°; 24-inch, -5.46°. These temperatures show, for January 2, a spread between the 2-inch and 24-inch levels of 12.48°.

During this same period, under a snow blanket of varying thickness, the soil temperature in no case fell below -1.0° C., the greatest spread between the 2-inch and 24-inch levels being only 3.5°, and at one time it was only 0.4°. By January 13 the snow had all disappeared and the temperature in both situations became more nearly similar, except for the slight lag due to the insulating effect of the sod. Even a slight fall of 0.5 of an inch of snow on January 30 was sufficient to effect a rise in the temperature of the 2-inch level, whereas the accompanying 3° drop in air temperature on that date was followed by a corresponding drop in the temperature of the 2-inch level under bare ground. Snow, therefore, even an inch or two, makes a remarkable difference in soil temperatures and, of course, in the chance of survival of the soil-inhabiting organisms, and may quite conceivably be a limiting factor in their ability to overwinter successfully.

EFFECT OF RAIN ON TEMPERATURE OF THE SOIL

Several hours' rain, even in frozen soil, had the effect of bringing the soil temperatures to equilibrium in the layers studied. That is to say, the temperature spread between the 2-inch and 24-inch layers was narrowed, in one case to 0.4° C. and in another to 0.3°. Cessation of the rain and the subsequent drying out of the soil resulted in a widening of the temperature gradient at the different depths.

Normally the soil moisture consists of hygroscopic and capillary water, the amount of the latter depending largely on the physical nature of the soil and its relation to the underlying water table. Since this is the case, the majority of the temperatures here discussed, being those of similar soil in practically the same locality, and with merely a surface-covering difference, can be compared directly as an indication of soil conductivity, the water being a constant factor. When, however, a rain of some hours' duration occurs there is introduced "free water," which, as it penetrates into the soil, mechanically destroys the temperature gradient which is one criterion of soil conductivity, and the soil temperatures then obtained are those of the free water modified by the specific heat of the soil and other intrinsic factors.

On February 7, 1 inch of rain fell during a 12-hour period, followed by a marked rise in air temperature. (Fig. 8.) The soil-temperature gradient in both situations studied practically disappeared. A considerable drop in air temperature immediately following this rise resulted in a corresponding drop in the soil temperatures, but owing to the fact that the sod covering in one instance slowed down evaporation, a difference in the temperature gradient resulted. This was most noticeable on the 25th of the month, when there was a difference of 4° in the temperature spread of the two situations. The bare ground, allowing of quicker evaporation, was colder.

During the spring months it will be noticed that any fairly heavy rainfall is followed by a narrowing of the temperature gradient in the soil. Even in midwinter a sharp thaw acts similarly even though the ground be frozen below the 2-foot level. In January from the 6th to the 13th the air temperature was above 0° C. and by the 12th there were water puddles everywhere from the melting snow. (Fig. 7.) The narrowing of the temperature gradient in the sod-covered ground is not striking but is evident nevertheless. The very noticeable effect on the bare-ground graph for this month may not be typical, as one corner of the plot was inundated, but similar conditions prevailed in every hollow and depression in the cultivated fields. Therefore, the introduction of free water in the form of melting snow or rain will cause a sudden sharp rise in soil temperature to the depth affected. This is again demonstrated in the graph for March (fig. 10) where heavy rain reduced the temperature spread in the first 2 feet to less than 0.5° . The effect of such comparatively rapid temperature changes on hibernating organisms is problematical.

THE CONDUCTIVITY OF SOILS OF DIFFERENT TYPES

It was desired to ascertain how the type of the soil affects its conductivity. Four types of soil having widely different characteristics were chosen. These were (1) a sandy loam from the experimental plot where these investigations were carried on; (2) a pure sand from the river bottom; (3) a silt loam from the experimental farm plots, and (4) a peat from the peat bogs of northern Minnesota. As these soils were so different in their physical properties and consequently in their water-holding capacity, the first question which arose was how to handle them so that the results of these conductivity experiments would be comparable. On the suggestion of C. O. Rost, of the division of soils of the Minnesota station, the writer used the

moisture equivalent⁴ as a basis of comparison of the temperature results. The moisture equivalent for each soil was determined to be as follows: Sandy loam, 7.39 per cent; river-bottom sand, 2.02 per cent; silt loam, 15.07 per cent; peat, 189.76 per cent.

Owing to faulty technic the results of this series of experiments were negligible and the experiments are mentioned merely to show the basis on which the work was conducted. Subsequent experiments at the Montana Agricultural Experiment Station indicate that the differences in soil conductivity are small. In those tests no attempt was made to control soil moisture. Holes of about a cubic yard capacity were dug in the ground outside and these were filled with loam, sand, and a sticky gumbo,⁵ respectively.

Daily temperature readings throughout the months of October, November, and December showed that the greatest difference ob-

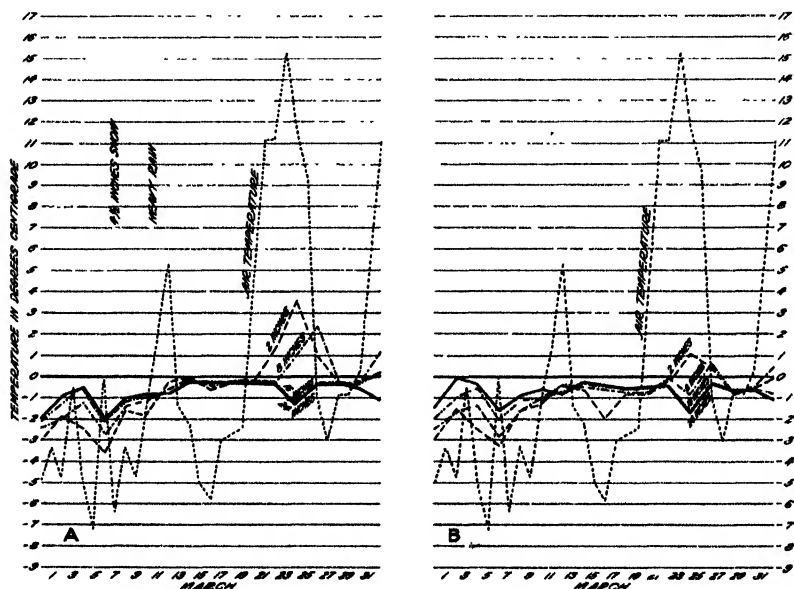


FIGURE 10.—Air temperatures and soil temperatures at different depths in bare (A) and sod-covered (B) ground in March

tained in the temperatures of the three soils at similar levels below 8 inches in depth was in no case greater than 2.2°C . (Tables 1, 2, 3.) The surface layers did show a larger spread, but this, from the standpoint of hibernating organisms, was not significant. A soil-temperature graph (fig. 11) for the period when the lowest temperatures prevailed at Bozeman, Mont., i. e., from January 15 to February 15, shows very clearly the slight differences between the temperatures of gumbo and sand at the same levels. Since moisture plays so important a rôle in the conductivity of the soil, very different results may be anticipated from a similar experiment in which soil is used at the moisture equivalent, but the conditions described are such as would be found in the field and are probably a more reliable indication of what one might expect to find in nature.

⁴The amount of water the soil will hold against a force of 1,000 times gravity.

⁵A fine colloidal clay containing practically no humus.

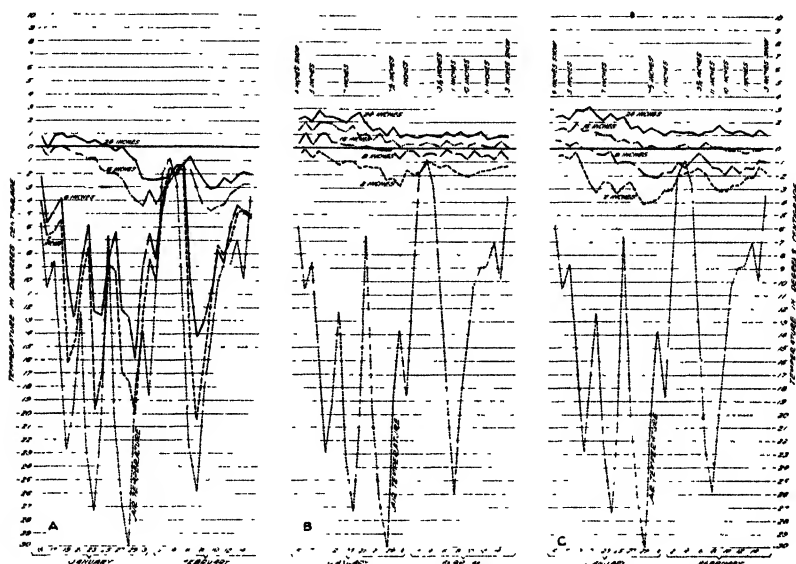


FIGURE 11.—Air temperatures and soil temperatures at different depths in bare ground (A), snow-covered gumbo (B), and snow-covered sand (C) at Bozeman, Mont., during the coldest part of the year

TABLE 1.—Daily temperature readings ($^{\circ}\text{C}$.) of different types of soils at different depths recorded during part of October at Bozeman, Mont., as compared with air temperatures

Date	2-inch depth			8-inch depth			16-inch depth			24-inch depth			Air temperature
	Loam	Gumbo	Sand	Loam	Gumbo	Sand	Loam	Gumbo	Sand	Loam	Gumbo	Sand	
Oct 11	1.9	3.8	3.3	7.0	8.2	7.3	10.2	10.2	10.3	10.8	10.9	11.4	2.8
Oct 12	0	7	8	3.8	5.0	4.1	7.8	7.6	7.2	8.5	8.5	9.1	2.3
Oct 13	2	5	7	3.7	4.6	3.7	8.2	7.8	7.2	8.7	9.3	9.3	2.5
Oct 14	2.7	3.6	4.6	4.5	5.6	5.2	8.8	7.7	7.6	9.8	9.0	9.2	3.5
Oct 15	3.4	4.2	4.6	4.7	5.6	5.7	7.9	7.7	7.8	9.0	9.0	9.6	4.2
Oct 16	4.2	4.1	4.3	5.0	5.6	6.0	7.3	6.9	7.9	8.0	7.8	9.1	4.6
Oct 17	9.9	7.4	8.3	8.0	8.0	8.8	9.3	8.7	9.4	9.3	9.3	10.0	5.0
Oct 18	2.3	3.2	3.4	5.6	6.4	6.4	7.6	7.1	8.1	8.0	7.4	8.5	4.4
Oct 19	2.3	4.3	4.8	7.2	8.1	8.1	9.4	9.3	9.9	9.6	9.3	10.3	4.9
Oct 20	5.3	5.3	6.2	7.5	7.5	7.6	9.2	7.7	8.7	9.5	8.2	9.0	4.7
Oct 22	6.0	4.3	5.0	4.0	4.3	4.5	7.25	6.0	6.6	8.0	7.5	7.5	4.6
Oct 23	5.3	1.6	4.5	6.0	6.1	6.5	8.0	7.6	8.5	8.2	8.3	8.9	5.0
Oct 24	3.5	3.3	3.4	5.6	6.5	6.5	7.8	7.6	8.0	7.9	7.5	8.1	4.4
Oct 25	3.2	2.9	3.5	5.1	6.1	6.1	7.6	7.3	7.8	8.2	8.0	8.6	4.5
Oct 26	1.6	2.6	3.0	5.2	6.2	6.4	7.6	7.3	8.2	8.1	8.2	9.0	4.5
Oct 28	1.3	2.0	2.0	3.6	5.1	5.8	7.4	7.0	8.1	8.3	8.0	9.3	3.2
Oct 29	2	.6	1.2	3.0	4.3	4.7	7.0	6.8	6.9	7.7	7.7	8.3	3.5
Oct 30	0	1.4	1.5	3.7	4.5	4.8	7.0	6.5	6.8	7.8	7.5	8.3	3.1
Oct 31	1.0	2.6	2.5	4.0	5.0	5.0	7.4	7.0	7.1	8.1	7.8	8.6	2.7
Average	2.83	3.23	3.55	5.11	5.63	5.96	8.04	7.57	8.00	8.60	8.38	9.06	3.91

TABLE 2.—Daily temperature readings (°C.) of different types of soils at different depths recorded during the month of November at Bozeman, Mont., as compared with air temperatures

Date	2-inch depth			8-inch depth			16-inch depth			24-inch depth			Air temperature
	Loam	Gumbo	Sand	Loam	Gumbo	Sand	Loam	Gumbo	Sand	Loam	Gumbo	Sand	
Nov. 1	-1.2	0	1.0	3.1	4.0	4.4	6.9	6.3	6.7	7.8	7.4	8.2	2.1
Nov. 2	-3.1	-1.0	-.9	1.2	2.9	2.3	5.1	5.2	5.1	7.0	6.6	7.0	2.2
Nov. 3	-1.3	0	3	2.1	3.2	3.0	6.0	5.6	5.4	7.0	6.8	7.5	2.9
Nov. 5	1.1	1.8	2	2.6	3.7	4.2	5.7	5.3	6.3	6.8	6.8	7.7	3.6
Nov. 6	1.0	2.1	2.0	2.6	3.3	3.5	4.8	4.7	5.0	6.0	5.7	6.8	3.6
Nov. 7	-1.1	.7	1.2	2.0	3.2	3.7	5.0	4.7	5.2	5.2	5.4	6.4	3.2
Nov. 8	-1.0	.2	1.1	1.5	2.7	3.0	4.2	4.5	4.9	5.1	5.3	6.0	3.5
Nov. 9	0	1.4	1.6	1.6	2.7	3.1	3.9	3.8	5.0	4.9	4.7	6.2	4.0
Nov. 10	4.9	4.2	4.8	4.8	4.8	5.2	5.6	5.4	5.9	6.2	6.0	6.9	4.8
Nov. 11	1.3	2.5	2.3	4.0	4.5	5.8	5.9	5.3	6.5	6.3	6.0	7.4	4.0
Nov. 12	-2	2.1	2.0	3.0	3.8	4.7	4.9	4.9	6.0	5.6	5.1	6.8	4.5
Nov. 13	5.0	4.9	5.6	5.3	5.1	5.9	5.8	5.4	6.6	6.0	5.8	7.1	4.7
Nov. 14	1.3	3.1	2.5	4.0	4.8	5.3	5.8	5.5	6.6	6.0	6.0	7.2	3.6
Nov. 15	3.7	2.8	2.8	2.8	4.1	4.3	5.4	5.7	5.5	6.2	6.0	6.3	3.5
Nov. 16	0	1.2	1.2	2.8	4.2	3.8	5.7	5.3	5.5	6.1	6.1	6.5	3.0
Nov. 17	0	1.7	1.6	2.2	3.8	3.3	5.4	5.3	5.0	6.2	6.2	6.4	2.6
Nov. 18	2	.1	.9	2.5	2.5	2.7	5.0	4.7	4.2	5.4	5.2	5.5	1.6
Nov. 19	0	3	8	1.7	2.8	2.7	4.5	4.7	4.5	5.3	5.3	6.0	2.6
Nov. 20	0	1.0	1.0	1.9	2.7	2.7	4.3	4.3	4.4	5.3	5.3	5.9	3.3
Nov. 21	.3	4	.8	2.1	2.7	2.1	4.4	4.1	4.2	5.2	4.9	5.5	3.9
Nov. 22	.8	.8	1.0	2.7	3.0	3.0	4.6	4.0	1.5	5.4	4.8	5.5	4.1
Nov. 23	2	.5	1.2	2.3	3.3	3.2	4.1	4.3	4.6	4.9	5.3	5.6	4.0
Nov. 24	4	.7	1.3	2.9	3.2	3.1	4.8	4.8	4.3	5.3	5.2	5.9	3.5
Nov. 25	1.2	1.8	1.2	1.7	2.3	2.5	4.1	3.7	4.2	5.0	4.6	5.3	3.6
Nov. 26	.6	1.3	1.2	2.3	3.9	3.0	4.5	4.6	4.6	5.2	5.1	5.6	3.8
Nov. 27	0	1.0	.9	1.8	3.0	2.8	4.5	4.7	4.8	5.2	5.0	5.9	2.9
Nov. 28	0	.5	.6	1.7	2.7	2.4	4.0	4.5	4.3	4.8	4.9	5.9	1.9
Nov. 29	-7	-2	0	1.0	1.8	2.1	3.5	3.6	3.7	4.4	4.4	5.1	1.5
Nov. 30	-7	-3	3	1.2	1.8	1.8	3.3	3.5	3.7	4.3	4.2	4.8	2.0
Average	.462	1.22	1.46	2.46	3.22	3.43	4.88	4.78	5.07	5.65	5.52	6.31	3.29

TABLE 3.—Daily temperature readings (°C.) of different types of soils at different depths recorded during the month of December at Bozeman, Mont., as compared with air temperatures

Date	2-inch depth			8-inch depth			16-inch depth			24-inch depth			Air temperature
	Loam	Gumbo	Sand	Loam	Gumbo	Sand	Loam	Gumbo	Sand	Loam	Gumbo	Sand	
Dec. 1	0	0	0	1.5	2.0	1.5	3.7	3.7	3.3	4.3	4.3	4.8	2.5
Dec. 2	-2	-1	0	.2	.1	.6	1.6	.6	.5	3.8	3.7	3.8	1.8
Dec. 3	-1.3	-4	-3	.6	1.3	1.4	3.1	3.0	3.0	3.8	3.9	4.3	.6
Dec. 4	-3.1	-7	-1.3	.5	1.2	1.2	3.3	3.0	3.0	3.8	3.7	4.2	1.6
Dec. 5	-2.8	-6	-1.3	0	1.3	.8	2.8	2.9	2.8	3.3	3.6	4.2	1.6
Dec. 6	-3.4	-6	-1.4	-.4	1.4	.9	2.8	3.0	2.7	3.8	3.8	4.3	1.8
Dec. 7	-4.9	-7	-1.9	-.9	1.2	.7	2.7	2.3	2.4	3.3	3.3	4.0	2.3
Dec. 8	-2.0	3	0	.6	2.5	2.1	4.3	4.2	4.3	4.8	4.8	5.7	2.8
Dec. 9	-4.0	-1.8	-2.4	-.9	0	0	1.1	1.0	1.0	1.7	1.6	1.6	3.0
Dec. 10	-3.7	-1.4	-2.3	-.3	0	0	1.3	1.4	1.3	2.9	2.5	3.0	3.2
Dec. 11	-1.0	-.6	-.3	-.4	.3	.5	2.1	2.2	2.0	3.0	2.8	3.6	3.1
Dec. 12	-1.4	-4	-1.5	.4	1.1	.2	2.7	2.4	2.0	3.2	3.2	3.9	1.6
Dec. 13	-.8	0	0	1.0	2.2	2.0	3.6	3.3	3.3	4.6	4.2	4.9	1.7
Dec. 14	-2.1	-.9	-1.1	-.9	.1	0	1.3	1.3	1.8	2.1	2.6	3.1	2.3
Dec. 15	-2.2	-1.0	-1.0	-1.0	0	0	1.1	1.2	1.2	2.3	2.3	2.6	1.8
Dec. 16	-1.4	-.9	-.7	.7	2	.1	1.5	1.5	1.4	2.3	2.5	3.1	1.6
Dec. 17	-1.7	-.8	-.8	-.6	.3	0	1.5	1.6	1.5	2.3	2.3	2.6	1.3
Dec. 18	-1.4	-.6	-.4	-.3	4	.3	1.7	1.9	1.8	2.4	2.5	3.2	1.4
Dec. 19	-1.2	-.2	-.3	0	8	.9	2.2	2.2	2.1	3.1	3.0	3.5	1.2
Dec. 20	-.3	-.1	-.2	.2	9	.8	2.2	2.1	2.1	2.9	2.8	3.5	1.6
Dec. 21	-1.6	-.7	-.9	-.5	.3	.1	1.7	1.3	1.3	2.5	2.4	2.9	1.9
Dec. 22	-2.6	-.8	-.9	-.5	.2	0	1.7	1.4	1.5	2.3	2.3	2.8	2.2
Dec. 23	-3.0	-1.4	-1.3	-1.4	-.5	-.5	1.2	1.2	1.0	2.0	2.1	2.4	1.9
Dec. 24	-2.3	-.7	-1.1	-.8	.3	2	1.9	1.5	1.5	2.3	2.4	2.8	1.8
Dec. 25	-1.3	-.7	-.5	-.4	.3	.2	1.4	1.6	1.7	2.2	2.3	3.0	3.0
Dec. 26	-2.3	-.7	-.7	-.6	.3	0	1.2	1.3	1.3	2.1	2.0	2.6	2.3
Dec. 27	-1.0	-1.0	-.3	-.4	1	0	1.5	1.5	1.3	2.1	2.2	2.5	4.0
Dec. 28	0	-.9	0	-.3	0	.3	1.3	1.1	1.6	1.9	1.8	2.8	3.6
Dec. 29	.8	0	.5	1.2	.6	1.0	2.5	1.8	2.0	2.9	2.7	3.3	3.1
Dec. 30	1.2	0	.3	1.1	.7	.7	2.3	1.8	1.7	2.8	2.5	3.0	2.9
Dec. 31	.9	.5	.9	1.3	1.4	1.3	2.7	2.4	2.6	3.2	3.2	3.7	2.0
Average	-1.61	-.57	-.687	-.5	.671	.54	2.09	1.99	1.96	2.8	2.78	3.31	2.15

Bouyoucos (1) showed that there is a remarkable equality in the specified heats of diverse types of soils and that this factor exercises practically no influence in causing and maintaining a difference in temperature in these various soils. It is the water content of the soil which is probably one of the chief intrinsic factors which might affect temperature variations. The average temperature of diverse classes of soils is not so variable as is popularly believed, and the well-known influence of color in affecting soil temperature is not here considered, as this factor can be largely discounted in winter on account of the snow.

INSECT SURVIVAL AT VARIOUS LOW TEMPERATURES

EFFECT ON WIREWORMS OF SUDDEN LOWERING OF TEMPERATURE

Many insect groups which are winter hardy have been found to have a lower freezing point in winter than they have in summer; in other words, they are able to withstand exposure in winter to temperatures which in summer would kill them. Payne (13) summarized the work done in this field by several workers, and demonstrated that the freezing point of oak borers becomes lower in winter and rises in the spring, and by summer is very near to that of water.

On July 17 several well-grown larvae of *Melanotus communis* Gyll. and *Ludius aeripennis*, Kirby var. *tinctus* were collected in the field and placed at a constant temperature of 5° C. At the end of 60 days one-third of these were dead, the remainder being still apparently healthy and active when the temperature was raised. A second batch of larvae of both species collected in summer and placed at a temperature of between 0° and 2° were all dead at the end of 14 days. This mortality was probably due to the fact that they were collected in summer, and, without being allowed to harden off by being submitted to a gradual lowering of the temperature, were placed immediately at a subnormal temperature for that time of the year. Since the mortality at 5° was so much smaller than at 0° to 2° it would appear that a range of a very few degrees above freezing is a critical point.

FREEZING POINT OF WIREWORMS IN LABORATORY EXPERIMENTS

Payne established the fact that wireworms (*Elater* sp.) are among the insects which undergo a hardening process. She noted that there is a considerable variation in the freezing points of individuals of any given species at a given time, or a periodicity in the freezing and undercooling points of the same species. This is borne out by the death of wireworms exposed suddenly to the relatively high temperature of 2° C. Gradual chilling, a few degrees at a time, results in the acquirement of a hardness which enables the insect to withstand extremes of cold, and Payne shows that the average freezing point of several larvae of the wood-borer, *Synchroa punctata*, chilled at -3° is decidedly lower than that of individuals of the same species which had not been chilled.

Using the technic described by Payne, the writer determined the freezing points of *Melanotus communis* larvae and adults. The hardening-off process apparently does not become pronounced until after exposure of the insect to temperatures lower than 8° C. This assumption is based on the fact that larvae which had been held at

constant temperatures of 32°, 27°, 22°, 17°, 12°, and 8° for some weeks showed little difference in their freezing and undercooling points. Also, the summer-collected larvae kept at 0° to 2° C. were all dead in 14 days. Of those kept at 5° approximately 66 per cent survived and among those kept at 8° there was no mortality.

TABLE 4.—Effect upon freezing and undercooling points of holding *Melanotus communis* larvae at different constant temperatures for some weeks

Larvae held at—	Average undercooling point of six larvae	Average freezing point for six larvae
°C.	°C.	°C.
32	-3.28	-0.79
27	-6.6	-.96
22	-6.36	-1.21
17	-7.11	-1.44
12	-7.33	-1.35
8	-5.93	-.99

The freezing points (shown in Table 4) were obtained by the piercing method, i. e., by puncturing the insect with the sharpened end of a thermojunction which was then forced into the tissues. The temperatures obtained seemed hardly logical in view of the conditions experienced by these larvae in nature and, in agreement with Carter (3), were not considered reliable. By the contact method, holding the couple in close contact with the insect, an average undercooling point of -14.6° C. and a freezing point of -12.8° were obtained. The averages by the piercing method were -6.1° and 1.11°, respectively.

The probable reason why freezing-point determinations made by the piercing method are unreliable is explained by Robinson (16, p. 751), as follows:

* * * When an insect is pierced a shock is produced within the organism which is accompanied by a flooding of the tissues with free water, due probably to the release of bound water held by the cell colloids. As a result, the lymph becomes diluted and the freezing point is raised.

In order to verify the possible accuracy of the piercing and contact methods, the following experiment was conducted. Several *Melanotus* larvae were placed in a Carrier temperature cabinet at a temperature of -2° C. Every 48 hours the temperature was lowered by 2 degrees, and at these times a larva was sectioned and examined microscopically for ice crystals. It was found that not until a temperature of -12° was reached were ice crystals present, which is in agreement with the freezing point of -12.8° determined by the contact method.

Similar discrepancies having been observed in determining the freezing points of the adult, a visual check was likewise run on 21 individuals. For the adults, by the piercing method, the average undercooling point was found to be -6.92° C. and the freezing point -3.16°. By the contact method the average undercooling point was -18.3° and the freezing point -15.5°. A correlation chart, such as is described by Robinson (16), was prepared for the adults of *Melanotus communis* to enable an approximation to be made of the natural undercooling and freezing points by contact. By using the figures -18.3 as the undercooling point and -15.5° as the freezing

point observed by the contact method, the chart shows the natural internal undercooling point to be -17.8° and the internal freezing point to be -14.8° . Several beetles from the same lot were, like the larvae, placed in the low-temperature cabinet and individuals removed at intervals for examination. The abdomen was cut in two with a pair of previously chilled scissors, and the tissue quickly examined under a binocular microscope. If the tissues were still fluid, the temperature in the cabinet was lowered by another degree. Observations are recorded in Table 5.

TABLE 5.—Effect of exposure to low temperatures for a period of six hours upon the adults of *Melanotus communis*

Temperature	Result
$^{\circ}\text{C}$	
-11	No sign of freezing
-12	Do
-13	Do
-14	Do
-16	Tissues frozen

The beetles used in these tests apparently froze between -14° and -16°C ., this being in close agreement with the internal freezing point as determined by the correlation chart. Several beetles were removed from the cabinet after 12 hours' exposure of -16° and allowed to thaw out at room temperature. In less than four hours they recovered their normal activity. A further series of temperature decreases to which the remaining beetles were subjected showed that they were able to withstand temperatures much lower than their undercooling point.

TABLE 6.—Ability of adults of *Melanotus communis* to recover their normal activity after exposure to low temperatures for different periods of time

Temperature	Period of exposure	Result
$^{\circ}\text{C}$	Hours	
-17	7	Recovered in less than six hours.
-18	12	Do.
-19	12	Do.
-21	12	Do.
-23	8	Did not recover.
-24	12	Do.

It would thus appear that the freezing point of *Melanotus communis* larvae is about -13°C . and that of the adults about -15° .

In this connection it is interesting to note that Fulton (6), working with the same species, found that the larvae were more resistant to heat than the adults, although, as stated above, the larvae are less resistant to cold.

FREEZING POINT OF WIREWORMS IN OUTDOOR CAGES

In connection with these hibernation studies it was desired to ascertain the freezing points of both adults and larvae of wireworms. As a preliminary to these freezing-point determinations, several larvae and adults of two species of wireworms, *Ludius aeripennis*, var.

tinctus and *Melanotus communis*, were buried at 2-inch intervals in the ground near the buried thermocouples. These wireworms were placed in salve tins previously well perforated with numerous small holes and filled with damp sand. Four cans were placed at each 2-inch interval from 2 to 24 inches in the fall of 1927 and dug up for examination on May 27, 1928. On examination it was found that the mortality was very low and obviously not due to temperature conditions. Of 49 larvae buried in the fall, 43 were found to be alive and active when dug up. The only beetle found dead was in a cage at the 4-inch level, the larvae in this cage also being dead, so probably their deaths were due to some local condition within the cage, as both adult and larvae at the colder 2-inch level survived. The dead larvae were from cans buried at depths of 4, 6, 10, and 12 inches, there being no deaths of either beetles or larvae at 2 inches. In the case of the 6 and 12 inch fatalities there were ants present and the larvae had been devoured, only the chitinous exuvium being left. However, it is improbable that the ants had killed the wireworms. All of the beetles which survived lived in the laboratory until the middle of July when they commenced to die off for want of attention. For food they were supplied with honey sirup.

Inasmuch as the lowest temperature to which these insects were exposed at 2 inches under snow was only -14.3°C . on January 28, and to that temperature but for one day, no mortality directly attributable to low temperature was to be expected.

McColloch, Hayes, and Bryson (11) in making hibernation studies on white grubs in Kansas, found that only 11.2 per cent of the 1,188 white grubs dug up were above the 6-inch level. Between 6 and 12 inches, 42 per cent were found, making 53.2 per cent of the grubs hibernating in the first 12 inches. Below the 24-inch level only 3.8 per cent were found.

H. L. Sweetman, working in Minnesota, found that white-grub adults have a much higher freezing point than wireworms. In beetles collected from the field on October 30, when presumably they had been subjected to some weeks of hardening, he found the undercooling point (by the contact method) to be -10.58°C . and the freezing point -6.5° . The average temperatures for several white-grub beetles with different previous histories were undercooling point -6.94° and freezing point -3.28° . In Table 7 are presented Sweetman's data (unpublished) showing the ability of June bugs, *Phyllophaga implicita*, to withstand low temperatures in their natural environment.

TABLE 7.—Ability of June bugs to withstand low temperatures when at various depths in the soil

Depth in soil	Number of adults—		Number of grubs—		Lowest tempera- ture
	Alive	Dead	Alive	Dead	
<i>Feet</i>					$^{\circ}\text{C}$.
0.5	0	9	0	2	-8.0
1.0	1	9	0	2	-6.75
1.5	3	7	0	2	-5.25
2.0	7	3	0	2	-3.75
4.0	10	0	1	1	0
5.0	10	0	2	0	0
6.0	6	4	2	0	0

It will be readily seen from these data that white grubs must of necessity hibernate at much lower depths than wireworms if they are to survive winter soil conditions, on account of their higher freezing points.

The freezing point of the larvae is not definitely known. It is apparently not much below 0° C. and in southern Minnesota they would, even under snow, have to go below 2 feet to overwinter successfully. Criddle (5) in Manitoba, records finding grubs which were hibernating at much greater depths than those recorded by McCulloch and Hayes in Kansas (9). From this it would appear that as one proceeds northward into colder climates the white grubs will be found hibernating at greater depths, and in southern Minnesota one would expect to find them at depths intermediate between those recorded from Kansas and Manitoba. If this be the case, then it would be conservative to assume that the majority of the Minnesota white grubs hibernate below 2 feet in depth. On this assumption, during the period of this study the minimum temperature to which they would have been exposed was above -6.9° , and under Minnesota conditions they would have adequate protection even in the entire absence of snow.

Normally the majority of wireworms, both adults and larvae, hibernate from 4 to 7 inches, although McCulloch, Hayes, and Bryson (10) show them as ranging from 3 to 36 inches, the majority being found at 7.7 inches during one winter and 10.1 inches during another. It has, however, been the observation of the writer, both in Alberta and Minnesota, that wireworms most often enter hibernation at a depth of from 5 to 7 inches. Be that as it may, wireworms, with their ability to withstand great extremes of temperature, will be amply protected from all danger of freezing in this latitude. Again referring to this season's temperature studies, it is apparent that even those wireworms which had entered into hibernation at a depth of 4 inches would only have experienced a minimum temperature of -17.8° C. which would probably be still within the safety zone for adults. This temperature would undoubtedly prove fatal to such of the larvae as were caught at 4 inches, their undercooling point being about -14.6° C., but those which were present at greater depths, and they are normally in the majority, would survive even bare-ground temperatures. Even as little as half an inch of snow affords sufficient assurance that the larvae will survive a normal Minnesota winter. It would require a prolonged spell of temperature in the neighborhood of -20° F. with an entire absence of snow, to destroy the wireworm population to any extent.

TO WHAT EXTENT DOES TEMPERATURE CONTROL WIREWORMS?

Wireworms do not build up and by their increase annually widen an area of infestation. Their long life cycle, from 3 to 5 years, if not longer, means slow multiplication. Infestations as a rule are local in character. A severely infested field or two may be found in one district and the nearest other infestation of importance may be many miles distant. This localization is, in the case of species the adults of which do not fly, e. g., *Ludius aeripennis* Kby., partly due to their restricted powers of locomotion. But some species are strong fliers and some other reason must be looked for to explain the

marked fluctuation in the incidence of this pest. Their known enemies are few. With few excepting predacious larvae and ground beetles can not, except in the case of very immature wireworms, pierce their thick chitinous cuticle. Some large *Calosoma* beetles, starved for several days and then liberated in a cage with several wireworms, avidly seized half-grown larvae in their mandibles but were unable to make the slightest impression. In the rearing cages fungous diseases attack the wireworms occasionally. A few worms in the laboratory died of a disease identified as *Oospora destructor* (Metch) Delacr. and healthy worms brought into contact with the diseased were also attacked and killed. Adults in the oviposition cages were killed by this same fungus. But the percentage of larvae or adults observed so killed in nature is infinitesimal. As to parasites, the only case which has come to the attention of the writer is an instance mentioned by Lane, who once obtained an unidentified parasite from a wireworm. Thomas (19), after a thorough review of the literature on parasites of wireworms, lists only 19 cases of insect parasitism and four annelid parasites, many of the observations not being well authenticated. On one or two occasions mites of the family Tyroglyphidae have been observed heavily infesting wireworms, but the hosts did not appear to be unduly inconvenienced apart from the mechanical irritation induced by their presence. Mite-infested wireworms have been kept for months and they developed normally and finally pupated. These are probably merely cases of indirect phoresy such as is mentioned by Wheeler (20). Bacterial disease undoubtedly takes a considerable toll of both wireworms and pupae, especially the latter. Dead and flaccid worms have been dug up occasionally, as well as several pupal cells containing a disintegrating mass. Birds, too, play their part in keeping down the number of wireworms, picking up many behind the plow. Pomerantzev (14) dissected out the stomachs of 120 rooks and found that insect pests and their larvae occurred to the extent of 55 to 76 per cent. Two species of wireworms, *Agriotes* and *Ludius*, were well represented among other beetles.

Climatic conditions then must undoubtedly be a control factor of much importance. In the summer when the larvae are active they can go down to escape fatal high temperatures, but as the soil cools they become dormant and a further drop in temperature after they become inactive finds them unable to escape. If the cold weather at this time is severe and sufficiently prolonged many of the wireworms will not survive. To a certain extent then, one may prognostigate from a study of the climatic conditions during the winter months whether such a pest as the wireworm is likely to be more, or less, severe during the ensuing spring. Wireworms are, according to King (8) among the major pests in Saskatchewan, and undoubtedly the heavy snowfall affords ample protection against the subzero weather which prevails for long intervals during most of the winter months in that Province. But it is not outside the bounds of possibility that a cold spell of several days' duration might follow a chinook,⁶ and under such circumstances a heavy wireworm mortality might reasonable be expected.

⁶ A warm sustained southwest wind common in parts of western North America, and which during the winter causes a rapid rise in temperature and removes all snow.

SUMMARY

A thermocouple apparatus for taking soil temperatures is described. Records of soil temperature during the winter months in Minnesota at depths from 2 to 24 inches are analyzed.

Snow is normally an adequate protection from cold to insects which hibernate at depths below 4 inches.

Rain or a rise in temperature inducing a thaw destroys the temperature gradient in the first 2 feet, and if such conditions are followed by a decided drop in temperature, hibernating insects may suffer high mortality.

The differences in the conductivity of soils of various types are not sufficiently great to be significant in insect hibernation.

Larvae and adults of *Melanotus communis* have a sufficiently low freezing point to withstand Minnesota winter temperatures if they hibernate below 4 inches in the ground.

Larvae and adults of two species of wireworms buried outside all winter at depths from 2 to 24 inches practically all survived—the small mortality not being due to temperature conditions. The possibility of larvae and adults of white grubs surviving similar conditions is discussed.

It is concluded that winter climatic conditions may in certain instances cause heavy mortality among hibernating wireworms.

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RELATION OF SIZE OF SWINE LITTERS TO AGE OF DAM AND TO SIZE OF SUCCEEDING LITTERS¹

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INTRODUCTION

The objects of this investigation were two: (1) To determine the relation of size of swine litter produced at a given age of the mother and the size of litters produced at later ages, and (2) to ascertain the relation of the size of a given litter to that of succeeding litters.

SOURCE OF MATERIAL

The data for this study were taken from the breeding and farrowing records of the swine herd of the department of animal husbandry of the University of Illinois, accumulated during the years 1903 to 1925. This investigation includes 935 litters containing 8,478 pigs of 7 breeds. The records were kept by the herdsman actively in charge of the barns. The temporary records of the herdsman were transferred to permanent records in the office, and from these the data for this study were taken. The breeds involved are Duroc-Jersey, Berkshire, Poland China, Chester White, Hampshire, Yorkshire, and Tamworth. The Duroc-Jersey, Berkshire, and Poland China comprise 24.4 per cent, 31 per cent, and 20.7 per cent, respectively, of the total number of litters studied. (Table 1.)

TABLE 1.—*Number of pigs, number of litters, and average size of litters studied*

Breed	Number of pigs	Number of litters	Average size of litter	Breed	Number of pigs	Number of litters	Average size of litter
Duroc-Jersey	2,265	228	9.93	Hampshire	472	52	9.07
Berkshire	2,112	290	8.42	Tamworth	455	45	10.11
Poland China	1,536	194	7.91	Total	8,478	935	9.06
Chester White	794	81	9.80				
Yorkshire	513	45	11.42				

OUTLINE OF THE STUDY

The litters were grouped into classes on the basis of the age of the dam, and the dams were classified on the basis of age at the time of farrowing, in intervals of six months, as follows: 10-15, 16-21, 22-27, 28-33, 34-39, 40-45, 46-51, 52-57, and up to 94-99.

The different phases of the study are as follows:

(1) The relation of the size of litter to the age of dam is shown for Duroc-Jersey, Berkshire, and Poland China breeds separately and for all three combined. (Fig. 1 and Table 2.)

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² The author is indebted to Dr. E. Roberts for helpful suggestions during the progress of this study.

(2) The average size of litter produced throughout the lifetime of the sow when first bred at an early age, and the average size of litter produced when the sow was first bred at a later age are shown for the three breeds separately and for the three breeds combined. (Table 3.)

(3) The correlation between the size of litter at a given age of the dam and the size of litter at later ages is shown for Duroc-Jersey, Berkshire, and Poland China breeds separately and for all the seven breeds combined. (Table 4.)

(4) The correlation between the size of one litter and the size of succeeding litters is shown for Duroc-Jersey, Berkshire, and Poland

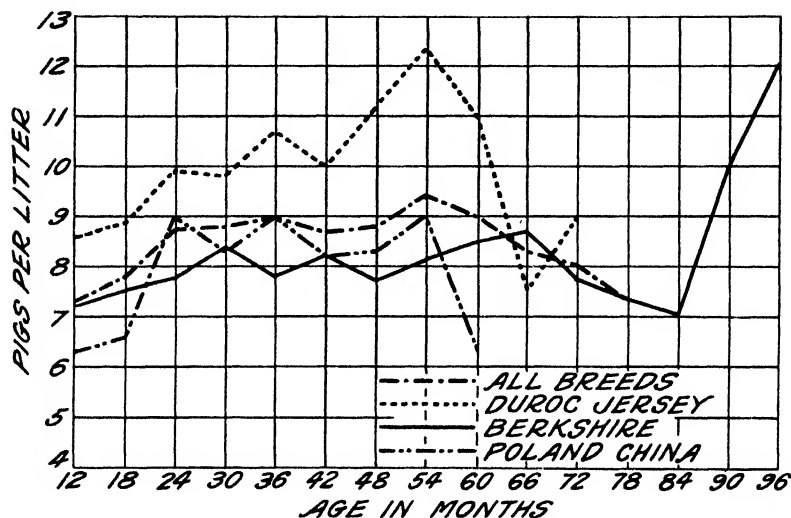


FIGURE 1. Size of litter farrowed at different ages of the sow. The figures on the abscissa represent average ages, for example, 12 represents the age of sows from 10 to 15 months, inclusive, 18, the age of sows from 16 to 21 months, inclusive

China breeds separately and for all the seven breeds combined. (Table 5.)

(5) The correlation between the size of the first litter and the average size of the combined second, third, and fourth litters produced by the same individual is shown, and also the correlation between the size of the first and the average size of the combined third and fourth litters produced by the same individual.

(6) The correlation between the size of the second litter and the average size of the combined third and fourth litters produced by the same individual is shown, and also the correlation between the size of the second litter and the average size of the combined third, fourth, and fifth litters produced by the same individual.

TABLE 2.—Data showing relation between age of dam and size of litter in Duroc-Jersey, Berkshire, and Poland China swine

DUROC-JERSEY							
Age of dam (months)	Number of pigs farrowed	Number of litters	Average size of litter	Age of dam (months)	Number of pigs farrowed	Number of litters	Average size of litter
12.....	309	36	8 58	48.....	113	10	11 30
18.....	312	35	8 91	54.....	62	5	12 40
21.....	435	41	9 89	60.....	41	4	11 00
30.....	216	25	9 84	66.....	15	2	7 70
36.....	245	22	10 68	72.....	9	1	9 00
42.....	130	13	10 00				
BERKSHIRE							
12.....	344	48	7 17	60.....	51	6	8 50
18.....	331	41	7 52	66.....	35	4	8 75
21.....	471	61	7 72	72.....	31	4	7 75
30.....	312	37	8 43	78.....	22	3	7 33
36.....	296	34	7 82	84.....	27	1	7 00
42.....	163	20	8 15	90.....	22	2	10 00
48.....	146	19	7 68	96.....	12	1	12 00
51.....	81	10	8 10				
POLAND CHINA							
12.....	252	40	6 30	42.....	106	13	8 15
18.....	115	22	6 59	48.....	108	13	8 30
21.....	300	10	9 00	54.....	45	5	9 00
30.....	192	24	8 35	60.....	13	2	6 50
36.....	180	20	9 00				
DUROC-JERSEY, BERKSHIRE, AND POLAND CHINA, COMBINED							
12.....	905	124	7 30	60.....	108	12	9 00
18.....	788	101	7 80	66.....	50	6	8 33
21.....	1,296	145	8 73	72.....	40	5	8 00
30.....	750	85	8 82	78.....	22	3	7 33
36.....	681	76	8 96	84.....	27	1	7 00
42.....	399	46	8 67	90.....	20	2	10 00
48.....	367	42	8 74	96.....	12	1	12 00
51.....	188	20	9 40				

TABLE 3.—Average size of litters from sows which farrowed first litter at 12 months compared with average size of litters from sows which farrowed first litters at 18 months

months

DUROC-JERSEY															Number of litters	Average size of all litters
Average size of litters when dam is aged (in months)																
12	18	24	30	36	42	48	54	60	66	72	78	84	90	96		
8 58	9 11	10 11	9 94	11 40	12 00	11 55	10 33	10 66	6 00	13 00					137	9 92
	8 70	9 92	10 86	9 44	11 00	8 00	9 33	13 00	9 50	9 00		4 00			65	9 60

BERKSHIRE															Number of litters	Average size of all litters
Average size of litters when dam is aged (in months)																
7 17	7 78	8 10	8 39	8 06	9 89	8 90	8 33	8 37	9 00	7 75	7 75	8 00	10 00	12 00		
	7 41	6 85	8 75	7 37	7 37	7 80	7 60	11 00	8 00	10 00	9 00	7 00		7 00	179	8 05
															90	7 51

POLAND CHINA															Number of litters	Average size of all litters
Average size of litters when dam is aged (in months)																
6 30	6 54	8 81	8 25	9 27	8 44	7 89	9 00	6 00								
	6 66	8 16	7 00	8 00	5 50	8 33	6 50	7 00							137	7 74
															28	7 18

DUROC-JERSEY, BERKSHIRE, AND POLAND CHINA, COMBINED															Number of litters	Average size of all litters
Average size of litters when dam is aged (in months)																
7 30	7 95	8 95	8 83	9 54	9 96	9 42	9 00	9 00	8 25	7 75	8 80	8 00	10 00	12 00		
	7 67	8 12	9 16	8 44	8 50	8 00	7 90	11 00	11 33	9 50	9 00	5 50		7 00	453	8 52
															183	8 24

TABLE 4—(Correlation between the size of litter at a given age of the sow and the size of litters at later ages)

DUROC-JERSEY							
Age of dam (months)	Number of litters	r	P E	Age of dam (months)	Number of litters	r	P E
10-15 and 16-21	19	-0.115	±0.154	22-27 and 28-33	21	+0.483	±0.112
10-15 and 22-27	26	+ .462	± .172	22-27 and 40-45	13	+ .662	± .105
10-15 and 28-33	16	+ .230	± .164	22-27 and 46-51	12	+ .395	± .127
10-15 and 34-39	17	- .225	± .155	28-33 and 34-39	17	+ .535	± .116
10-15 and 46-51	9	+ .612	± .140	34-39 and 40-45	15	+ .780	± .068
16-21 and 22-27	23	+ .097	± .112	34-39 and 46-51	13	+ .500	± .046
16-21 and 28-33	14	+ .298	± .164	40-45 and 46-51	11	+ .611	± .127
16-21 and 34-39	17	- .089	± .162				
BERKSHIRE							
10-15 and 16-21	15	+0.090	±0.172	22-27 and 28-33	23	-0.249	±0.131
10-15 and 22-27	33	+ .680	± .117	22-27 and 34-39	25	- .032	± .134
10-15 and 28-33	22	+ .0005	± .143	22-27 and 46-51	15	- .194	+ .167
10-15 and 34-39	19	+ .226	± .148	22-27 and 52-57	10	+ .416	± .176
10-15 and 40-45	10	+ .201	± .205	28-33 and 34-39	22	- .095	± .142
16-21 and 22-27	22	+ .330	± .128	28-33 and 40-45	12	+ .409	± .163
16-21 and 28-33	11	- .315	± .183	28-33 and 46-51	13	+ .548	± .132
16-21 and 34-39	12	- .019	± .197	34-39 and 46-51	16	+ .070	± .165
POLAND CHINA							
10-15 and 16-21	13	+0.684	±0.099	16-21 and 22-27	14	+0.651	±0.103
10-15 and 22-27	30	+ .472	± .096	16-21 and 28-33	10	- .056	± .212
10-15 and 28-33	15	+ .064	± .170	22-27 and 28-33	21	- .343	± .129
10-15 and 34-39	15	- .449	± .137	22-27 and 34-39	11	+ .191	± .036
ALL BREEDS COMBINED *							
10-15 and 16-21	62	+0.229	±0.080	22-27 and 34-39	92	+0.277	±0.064
10-15 and 22-27	114	+ .313	± .055	22-27 and 40-45	52	+ .303	± .085
10-15 and 28-33	71	+ .125	± .077	22-27 and 46-51	45	+ .253	± .097
10-15 and 34-39	81	+ .229	± .071	22-27 and 52-57	22	+ .508	± .106
10-15 and 40-45	38	+ .653	± .063	28-33 and 34-39	73	+ .321	± .071
10-15 and 46-51	33	+ .115	± .115	28-33 and 40-45	45	+ .526	± .072
10-15 and 52-57	17	- .229	± .155	28-33 and 46-51	39	+ .477	± .098
16-21 and 22-27	77	+ .440	± .062	28-33 and 52-57	21	+ .197	± .141
16-21 and 28-33	54	+ .363	± .079	34-39 and 40-45	53	+ .425	± .085
16-21 and 34-39	46	+ .037	± .099	34-39 and 46-51	51	+ .415	± .078
16-21 and 40-45	26	+ .018	± .130	34-39 and 52-57	24	+ .389	± .168
16-21 and 46-51	18	+ .396	± .134	40-45 and 46-51	40	+ .603	± .067
16-21 and 52-57	11	+ .161	± .197	40-45 and 52-57	25	+ .294	± .123
22-27 and 28-33	90	+ .139	± .069	46-51 and 52-57	23	+ .580	± .140

* Duroc-Jersey, Berkshire, Poland China, Chester White, Hampshire, Yorkshire, and Tamworth.

TABLE 5.—Correlation between the size of one litter and size of succeeding litters

DUROC-JERSEY							
Litters correlated	Number of litters	r	P E	Litters correlated	Number of litters	r	P. E.
1 and 2	53	+0.245	±0.087	2 and 5	15	+0.055	±0.173
1 and 3	37	+0.280	±0.102	2 and 6	10	+0.515	±0.120
1 and 4	28	+0.040	±0.127	3 and 4	25	+0.381	±0.115
1 and 5	19	+0.052	±0.154	3 and 5	16	+0.633	±0.101
1 and 6	11	+0.016	±0.204	3 and 6	12	+0.309	±0.167
2 and 3	35	+0.392	±0.096	4 and 5	17	+0.442	±0.131
2 and 4	25	+0.228	±0.123	4 and 6	11	-0.009	±0.203
BERKSHIRE							
1 and 2	81	+0.178	±0.073	2 and 6	13	+0.617	±0.115
1 and 3	44	+0.013	±0.102	3 and 4	27	+0.207	±0.120
1 and 4	27	+0.361	±0.128	3 and 5	20	+0.412	±0.118
1 and 5	21	+0.709	±0.523	4 and 5	21	+0.087	±0.146
1 and 6	13	+0.078	±0.181	4 and 6	13	+0.216	±0.180
2 and 3	41	+0.117	±0.104	4 and 7	9	-0.408	±0.185
2 and 4	27	+0.119	±0.124	5 and 6	13	-0.311	±0.186
2 and 5	19	0.373	±0.133				
POLAND CHINA							
1 and 2	43	+0.426	±0.081	2 and 4	20	+0.184	±0.099
1 and 3	29	+0.418	±0.103	2 and 5	12	+0.145	±0.176
1 and 4	19	+0.182	±0.150	3 and 4	20	+0.163	±0.145
1 and 5	11	+0.087	±0.201	3 and 5	11	+0.603	±0.133
2 and 3	31	+0.450	±0.096				
ALL BREEDS* COMBINED							
1 and 2	228	+0.341	±0.030	2 and 7	19	+0.445	±0.139
1 and 3	150	+0.232	±0.052	3 and 1	96	+0.367	±0.059
1 and 4	94	+0.207	±0.064	3 and 5	65	+0.542	±0.059
1 and 5	67	+0.248	±0.071	3 and 6	42	-0.069	±0.100
1 and 6	40	+0.256	±0.099	3 and 7	22	+0.306	±0.130
1 and 7	22	-0.355	±0.140	3 and 8	14	+0.524	±0.136
1 and 8	14	0.144	±0.176	4 and 5	65	+0.441	±0.067
2 and 3	189	+0.367	±0.042	4 and 6	42	+0.022	±0.104
2 and 4	93	+0.248	±0.066	4 and 7	22	+0.438	±0.130
2 and 5	68	+0.155	±0.079	4 and 8	13	+0.578	±0.120
2 and 6	41	+0.664	±0.058	5 and 6	40	-0.065	±0.106

* Duroc-Jersey, Berkshire, Poland China, Chester White, Hampshire, Yorkshire, and Tamworth

ANALYSIS OF DATA

The average size of all litters involved is 9.06 pigs (Table 1), as compared with 8.1 found by Carmichael and Rice.³ The average size of litter in present work was determined by including litters only from dams that produced more than one, all dams producing only one being eliminated. This fact may account for the higher average found in this study as compared to that reported by Carmichael and Rice.

Rommel,⁴ working with registration records, found the average size of litter for Duroc-Jerseys over a period of 10 years (1893-1902) to be 9.26 pigs and the average size of litter for Poland Chinas over

³ CARMICHAEL, W. J., and RICE, J. B. VARIATIONS IN FARROW* WITH SPECIAL REFERENCE TO THE BIRTH WEIGHT OF PIGS. Ill. Agr. Expt. Sta. Bul. 226, p. 67-95, illus. 1920

⁴ ROMMEL, G. M. THE FECUNDITY OF POLAND CHINA AND DUROC-JERSEY SOWS. U. S. Dept. Agr., Bur. Anim. Indus. Circ. 95, 12 p. 1906.

a period of 5 years (1898-1902) to be 7.52 pigs. In the present investigation the average size of the Duroc-Jersey litters was found to be 9.93 pigs and the average size of the Poland China 7.91.

There is a marked increase in size of litter with increasing age of the dam until about 4½ years. (Fig. 1 and Table 2.) All breeds combined show an increase in size of litter from 7.3 pigs at 1 year to 9.4 pigs at 4½ years, after which there is a gradual decrease to 7 pigs at 7 years.

Carmichael and Rice⁵ found that the average size of litters of all breeds combined reached its peak at 3 years instead of 4½ as found in this study. This may be due to the ration and management of the herd, and the selection of more prolific individuals since the work of Carmichael and Rice.

Hammond⁶ concludes from an investigation of the number of corpora lutea present in the ovaries and the number of fetuses present in the uteri that the lower fertility of young sows is due to the formation of a smaller number of ova.

Is the average size of litter during the reproductive life of the sow affected by the age at which she is first bred? Data are presented in Table 3 showing the difference in size of litter produced by sows bred at different ages. Since no difference was found among breeds in this respect, records of Duroc-Jerseys, Berkshires, and Poland Chinas were combined. (Table 3.) The litters from sows that produced only one litter are eliminated. The difference is determined between the average size litter of the group that farrowed their first at 12⁷ months and the average size litter of the group that farrowed their first at 18⁸ months. The average of the sows that farrowed their first litter at 12 months was 8.52 pigs, based on 453 litters. An average of 8.24 pigs per litter for the group of sows that farrowed their first at 18 months was obtained, based on 183 litters. The difference 0.28 ± 0.158 is not significant.

Because of the great variation in size of first litters, these were eliminated in determining the difference of the average of each group. An average of 8.98 pigs per litter for the group that farrowed the first litter at 12 months was obtained, based on 329 litters. The average of the group that farrowed their first at 18 months was 8.49 pigs per litter, based on 127 litters. The difference 0.49 ± 0.173 is not significant.

Evidently breeding sows at an early age does not increase their fertility. However, no evidence was found to show that breeding sows as young as possible lowers fertility. On the other hand, early breeding makes possible a greater total number of pigs during the breeding life of the sow and also has the advantage of enabling the breeder to detect barren females earlier than would otherwise be the case.

The number of litters involved in the group in which the litters were farrowed at 2 years of age are so few that no conclusion can be drawn.

In Table 4 are shown coefficients of correlation between the size of litter at a given age of the sow and size of litter at later ages. A

⁵ CARMICHAEL, W. J., and RICE, J. B. Op. cit.

⁶ HAMMOND, J. ON SOME FACTORS CONTROLLING FERTILITY IN DOMESTIC ANIMALS. *Jour. Agr. Sci. [England]* 6: 263-277, illus. 1914.

⁷ Farrowed in the interval between 10 and 15 months, inclusive.

⁸ Farrowed in the interval between 16 and 21 months, inclusive.

marked correlation ($r = +0.313 \pm 0.055$) is observed between the size of litters farrowed at 1 year and the size of litters at 2 years in which 114 litters are involved.

There is also a significant correlation between the size of litter at 2 years and the size of litter at $4\frac{1}{2}$ years, $r = +0.508 \pm 0.106$; between $2\frac{1}{2}$ and $3\frac{1}{2}$ years, $r = +0.526 \pm 0.072$; between $2\frac{1}{2}$ and 4 years, $r = +0.477 \pm 0.098$; between 3 and $3\frac{1}{2}$ years, $r = +0.425 \pm 0.085$; between 3 and 4 years, $r = +0.415 \pm 0.078$; between $3\frac{1}{2}$ and 4 years, $r = +0.603 \pm 0.067$.

The numbers involved in the correlations between the size of one litter and the size of the succeeding litters in Duroc-Jerseys, Berkshires, and Poland Chinas separately are, in general, too small to be significant. (Table 5.)

Among all breeds a significant correlation is observed. (Table 5.)

Between the first and second litters, $r = +0.341 \pm 0.030$.

Between the first and third litters, $r = +0.232 \pm 0.052$.

Between the first and fourth litters, $r = +0.267 \pm 0.064$.

Between the second and third litters, $r = +0.367 \pm 0.042$.

Between the second and sixth litters, $r = +0.6664 \pm 0.058$.

Between the third and fourth litters, $r = +0.367 \pm 0.059$.

Between the third and fifth litters, $r = +0.542 \pm 0.059$.

Between the fourth and fifth litters, $r = +0.441 \pm 0.067$.

Between the fourth and eighth litters, $r = +0.578 \pm 0.120$.

The correlation between the size of the first litter and the average of the second, third, and fourth with 98 litters involved was found to be $r = +0.232 \pm 0.065$; the correlation between the size of the first litter and the average of the second, third, fourth, and fifth with 67 litters involved was found to be $r = +0.195 \pm 0.079$; the correlation between the size of second and the average of the third and fourth with 98 litters involved was found to be $r = +0.409 \pm 0.059$; and the correlation between the size of second and the average of the third, fourth, and fifth with 67 litters involved was found to be $r = +0.290 \pm 0.075$.

The correlation between the size of the second litter and the average size of succeeding litters is more significant than the correlation between the size of the first and the average size of succeeding litters. The correlation between the size of the first litter and the average size of succeeding litters is less than four times the probable error; while the correlation between the size of second litters and the average of succeeding litters is more than four times the probable error.

The higher coefficient of correlation obtained between the second and succeeding litters is due to the greater variation in the size of first litters. The greater variation in the size of the first litter is clearly shown in the calculation of the coefficients of variability of the first and second litters involved in the correlation. (Table 6.)

TABLE 6.—Coefficient of variability of the first and second litters

Litter	Number of litters	Coefficient of variability	Probable error
First.....	98	36.64	± 1.5
Second.....	98	28.95	± 1.3

SUMMARY AND CONCLUSIONS

In general the size of litter increases with the age of the dam up to about 4½ years, after which a gradual decrease occurs.

The fertility of a sow is not affected by the age at which she is first bred, but the results of this investigation show that it is advisable to breed the sow young, since more pigs will be produced throughout her breeding life. Moreover, early breeding makes possible early elimination of barren individuals.

A significant correlation exists between the size of litter farrowed at a given age of the dam and the size of litter farrowed at later ages of the dam.

Where sufficiently large numbers of litters were available for study significant correlations were found between the size of a given litter and the size of succeeding litters.

A high correlation exists between the size of the second litter and the average size of later litters.

The coefficient of variability in size of first litters is greater than in the size of second litters.

This study shows that size of litter is a valuable criterion in selection for fertility.

EFFECT OF CEANOTHUS BRUSH ON WESTERN YELLOW PINE PLANTATIONS IN THE NORTHERN ROCKY MOUNTAINS¹

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INTRODUCTION

Forest planting in the northern Rocky Mountain region is largely confined to areas that have been burned over twice, the second burning occurring during recent years. Planting crews can operate on these "double burns" with relative ease because the fires have removed shrubs and other obstructions. Unfortunately planting activity on forest lands is not keeping pace with the accumulation of areas denuded by fire, and as time passes these lands rapidly revert to shrubby growth from the uninjured roots of the original bushes and from wind-borne seeds. Released from competition, other than that of herbaceous plants, the bushes frequently usurp the area. This is especially true of *Ceanothus velutinus* on southerly slopes. Certain areas are considered "too brushy to plant" because of the mechanical hindrance of the bushes. On other areas those in charge of this work feel uncertain as to whether or not the beneficial effect of shade from the bushes is greater than the detrimental effect of root competition. Hence, when confronted with a bush in his path, the planter lacks instructions about the proper location of his seedlings. Each planter, therefore, follows the line of least resistance by avoiding the bushes as much as possible and setting his trees in the intervening spaces. A desire for definite information as a basis for improved practice led to the experiments reported here.

EARLY WORK WITH VARIOUS SPECIES

Observations made in other regions on the effect of various shrubs on conifer seedlings may be mentioned briefly. As early as 1912 Kimball and Carter (6),² working with white pine in Massachusetts, observed that medium or heavy low shade with root competition appeared preferable to no shade and no root competition. Later Hofman (5), working in the Cascade Mountain region, concluded that shade was not an important factor in reforestation except when the site is very poor or the shade very dense. Still more recently Show (10), working principally with western yellow pine in California, reached these conclusions:

1. Brush cover increases survival, and the benefit of shade is greater on poorer sites, for less drought-resistant species, and for poorer stock.
2. The quality of work done in planting improves as the brush cover decreases.
3. The influence of different species of brush is found to vary greatly. *Manzanita* is least detrimental to success in planting.
4. Increased shading results in increased height growth.

¹ Received for publication June 13, 1930, issued October, 1930.

² Reference is made by number (italic) to Literature Cited, p. 612

From the early forest planting work in northern Idaho and western Montana it was evident that trees planted in the shelter of stumps and fallen logs usually survived better than those set in more exposed spots. The difference in moisture conditions seemed to be what affected the trees. These casual observations were verified by a special test in the spring of 1923 in which 800 trees were planted and half of them sheltered by shingles driven into the ground close to each plant on the sunny side. Two hundred western white pines (*Pinus monticola*) on a northwest slope and a similar number of western yellow pines (*P. ponderosa*) on a southwest slope were sheltered in this way. Survival of both species of trees was higher when sheltered than when in the open. It seemed natural to expect that the effect of brush on trees, so far as the tops only were concerned, should be similar to the effect of the logs, stumps, and shingles. That the adverse effect of root competition might more than offset the benefit of shelter was suggested by the results of another test made

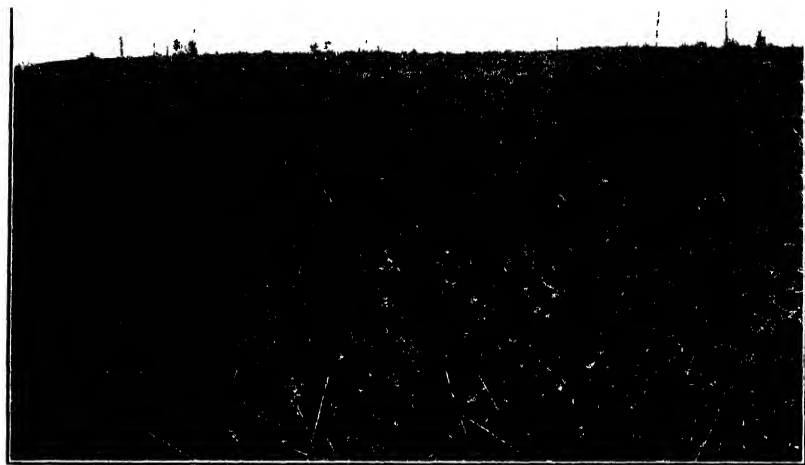


FIGURE 1.--East slope bearing a scattered stand of *Ceanothus velutinus*. This was the site of the test plantation

that year (1923). Trees planted under bushes of various species and sizes did not do so well as similar ones planted in the open. No conclusions were possible, however, because of the small number of trees and the lack of adequate experimental control. These preliminary tests indicated the need for more intensive experiments.

EXPERIMENTS WITH YELLOW PINE AND CEANOTHUS

INSTALLATION OF TESTS

In the spring of 1926 a mountain slope, considered typical of much of the land in need of planting in the region (fig. 1), was selected for the experiment, near Haugan, Mont. It was a portion of the area burned in 1910 and constituted a uniform easterly slope bearing an evenly scattered stand of *Ceanothus velutinus*. The bushes consisted of numerous stems branching from the root collar, at which point the oldest stems were found to range from 10 to 14 years of age. However, most of the stems were found to be only about half as old

and in their more or less sprawling position rose only from 2 to 4 feet from the ground. In this experiment it was desired to study the effect of brush on western yellow pine, independently of shade from other objects. Accordingly the area selected was one having a minimum number of dead trees either standing or down.

The trees for planting were taken from the general run of 1-2 western yellow pine stock at Savenac nursery after the most poorly developed specimens had been rejected, a number amounting to 13½ per cent of the total. Roots were pruned to a length of 8 inches. Six hundred bushes well distributed over the mountainside were selected, and three trees were planted (April 9-13, 1926) on the slope just below each bush. One tree was placed well under where it received much shade and root competition; one was placed well out where it received no root competition from bushes and very little, if any, shade; and the third tree was set in an intermediate position where it received some shade and doubtful root competition. This intermediate position was under the outer edge of the crown of each bush. All trees were planted by the same man in a uniform manner, using the method standardized for Forest Service planting in the region.

ENVIRONMENTAL FACTORS WORTHY OF STUDY

Apparently inconsistent results are often obtained from experiments with plants simply because of a dearth of information concerning some of the factors involved. However, the multiplicity of these factors and the impossibility of studying them all at any one time or place, necessitates the attempt to measure only a few and to control or eliminate the influence of others. In this experiment only the factors believed to be of most vital importance were studied. Years of experience indicate that of all environmental factors, moisture relations are the most influential on the life of planted trees. This was recognized by Korstian (?) working with western yellow pine in Utah. Although light was not studied in this experiment, because of the lack of suitable instruments and the belief that its rôle is subordinate to that of moisture, a study was made of the more readily measurable, and probably more significant, temperature relations.

When soil-moisture determinations can be expressed in terms of moisture available to the crop studied, their significance can be much more readily interpreted than when they are expressed in terms of total moisture. Accordingly, from several points scattered over the site of the test plantation, soil samples were taken to represent the upper 8-inch layer of soil. This earth was then used in the greenhouse for a determination of the wilting coefficient for western yellow pine. The method used was that advocated by Bates and Zon (3). The weighted averages employed in this method gave these results: Pan No. 1, 4.1 per cent; pan No. 2, 3.6 per cent; or an average, approximately, of 3.9 per cent, as the wilting coefficient. This figure was then applied to the results of field and laboratory determinations of total moisture, expressed in percentage of oven-dry weight, and shown in Table 1.

The death of plants from drought is due not only to the lack of available soil moisture but almost equally to the loss of moisture through transpiration of the tops. The external factor most directly

affecting transpiration is evaporation, depending as it does on the combined influence of wind, temperature, and humidity, each of which is recognized as an important factor in the survival of plants during periods of drought. Accordingly, a study of evaporation was made involving the locations of the western yellow pines planted under *Ceanothus*. A typical bush was selected near the center of the plantation and two Bates evaporimeters were placed underneath, two in



FIGURE 2.—View of instruments as installed for a study of evaporation and temperature in relation to *Ceanothus* brush

the open space on the slope below, and one at the intermediate position under the edge of the crown. (Fig. 2.) A continuous record of evaporation at these three points was thus obtained for the 81 days from June 17 to September 6. Thus the figures given in Table 2 have a sufficient basis to show the average relative rates of evaporation under and away from the bushes during the summer period.

No thorough study was made of atmospheric humidity as an independent factor. Theoretically absolute humidity, because of

transpiration from the foliage and evaporation from the moister soil, would be greater under the bushes than in the open. This condition, and also the lower air temperature in the shade, would cause a still greater contrast in relative humidity under the brush and outside. Such a contrast, if it actually exists over small areas, would naturally be greatest when the sun shines and no wind is blowing. Direct measurements of relative humidity with a cog psychrometer on two calm and sunny days gave the figures shown in Table 3. Undoubtedly average conditions, had they been determined, would show similar though less marked contrasts.

TABLE 1.—*Soil moisture in relation to Ceanothus*

[Moisture in percentage of oven-dry weight of soil]

Date	Position relative to brush	Basis (samples)	Total moisture	Wilting coeff- cient	Available moisture
		Number	Per cent	Per cent	Per cent
1926					
July 7	Open	7	6.2	3.9	2.3
Do	Intermediate	1	9.1	3.9	5.2
Do	Under	7	9.4	3.9	5.5
Aug 7	Open	7	3.5	3.9	— .4
Do	Intermediate	1	3.2	3.9	— .7
Do	Under	7	4.5	3.9	— .6

TABLE 2.—*Evaporation in relation to Ceanothus*

[Averages per 24-hour day]

Dates	Period	Position relative to bush		
		Under	Inter- mediate	Open
1926	Days	Grams	Grams	Grams
June 17-June 29	11 8	5	7	11
June 29-Aug 3	35 0	6	10	15
Aug. 3-Sept. 6	34 2	4	6	7
June 17-Sept 6	81 0	5	8	11

TABLE 3.—*Relative humidity in relation to Ceanothus (read from cog psychrometer)*

Date	Position relative to bush		
	Under	Inter- mediate	Open
1926	Per cent	Per cent	Per cent
July 2	62	48	47
July 6	44	40	33
Average	53	44	40

That soil temperatures are influential in plant life is generally recognized. The influence of forest cover on soil temperature has been studied recently by Li (9). He found that the forest cover near Keene, N. H., reduced the maxima, minima, and means of soil temperatures at all depths through the summer. This forest cover consisted of a canopy of trees and the litter of the forest floor. Brush

cover might reasonably be expected to have a similar, though less marked, effect. Records were obtained by the simultaneous operation of two carefully adjusted soil thermographs with soil bulbs extending from the surface to a depth of 8 inches, one under brush, the other in an adjacent opening. These thermograph traces form the basis for the graph shown in Figure 3. Apparently the brush had a marked effect in lowering soil temperature and reducing its range within the upper 8 inches where the planted roots are. Bates (2) emphasizes the importance of high temperatures at the surface of the soil, and Toumey (11) found that the surface temperatures varying from 122° to 131° F. killed very young, tender conifer seedlings. Although such high temperatures might not be actually fatal to the 3-year-old trees planted in these tests, it was thought that they might easily affect thrift and early growth. Thermometers observed during the period from June 17 to September 6 indicated the following maximum

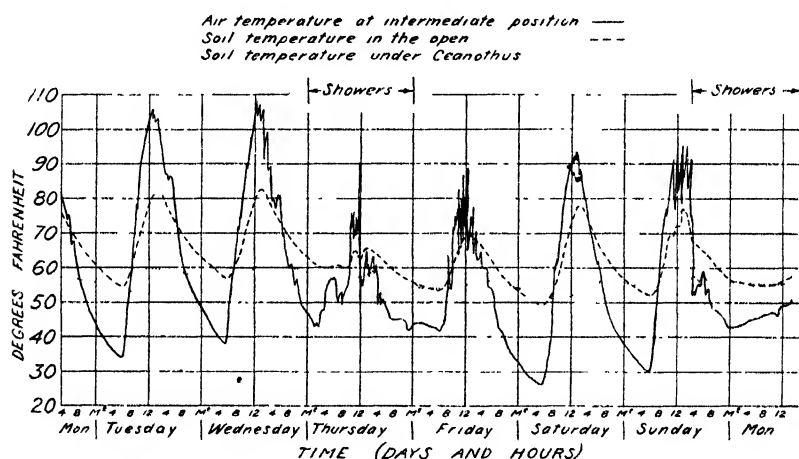


FIGURE 3.--Thermograph tracings showing relations of air temperatures and soil temperatures to a depth of 8 inches on planted area during last week of August, 1926

surface soil temperatures: Under Ceanothus 94°, intermediate position 138°, and open position 145°.

EXAMINATION OF TREES

When the western yellow pines planted in the Ceanothus brush field were examined in August, 1926, the foliage of the current year's growth on shaded trees was strikingly different from that on the unshaded ones. Under Ceanothus the needles on new shoots were longer, thinner, and more delicate in appearance than those grown in the open. The needles within each fascicle on shaded trees were well separated from each other in the normal way, whereas on trees standing in the open the new needles had not separated. These open-grown needles were very noticeably shorter and thicker than those grown in the shade, although not as thick as the long, mature western yellow pine needles from older trees. These differences in external appearance of shade-grown as compared with open-grown needles were so marked that it was thought worth while to look for corresponding differences in internal structure. Korstian (8), working

with 3-year-old Douglas fir (*Pseudotsuga taxifolia*) seedlings in seed beds at the Cottonwood nursery in Utah, found that the leaves of shaded plants had a less compact structure, thinner epidermis and cuticle, more spongy tissue, closer lying cells, and less deeply depressed stomata than plants growing in the open sunlight. Laboratory study of the yellow pine needles failed to reveal any such significant differences in cell structure, but it served to illustrate more clearly the relative size of the needles as a whole. Figure 4 shows that the shade-grown needles, although apparently healthy and normal in

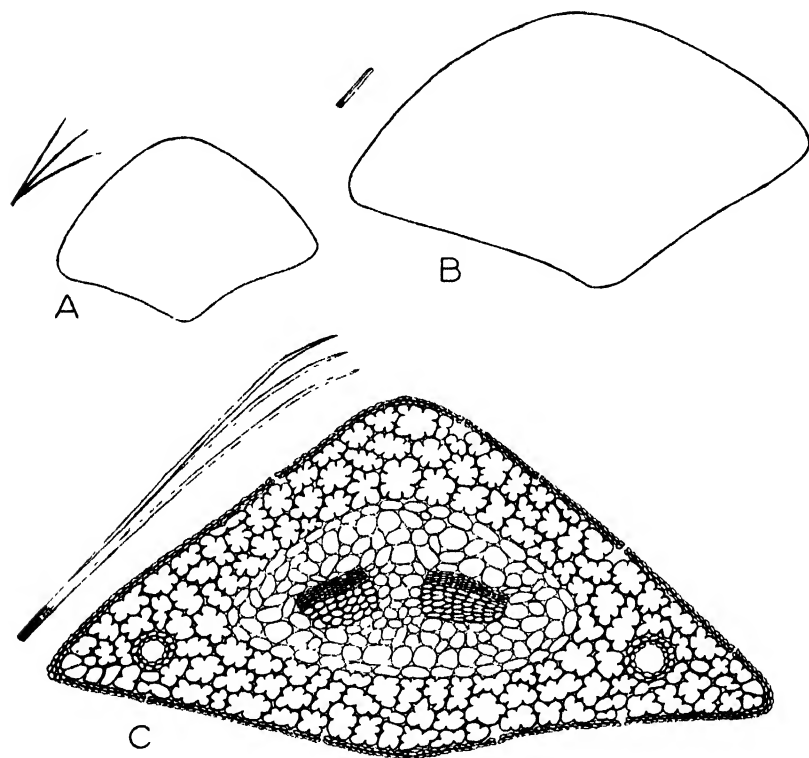


FIGURE 1. The diagram shows the relative size of needle fascicles (one-half actual size) and cross sections of needles (magnified seventy-five times) from 3-year-old western yellow pine transplants planted (A) under a *Ceanothus* bush and (B) in the open. The figure also shows (C) fascicles and cross section of a 13-year-old, naturally seeded, open-grown, western yellow pine from the same area. The internal structure of A and B is similar to that shown for C.

structure, were much more slender and delicate than the needles from an older open-grown and naturally seeded yellow pine. The needles from the tree planted in the open were intermediate in thickness and doubtless would also have been intermediate in length had it not been for the action of drought. Obviously drought caused a cessation of development in these new needles, leaving them short and closely appressed in the fascicle, as shown in the drawing. This did not happen to the new pine needles under the *Ceanothus* because of the greater soil moisture and less desiccating atmospheric conditions existing there, as indicated by the above tables.

It was evident that under the bushes evaporation was less, and that, at least during periods of little wind and much sunshine, humidity was higher than in the open. Furthermore, as indicated in Table 1, by the end of the first week in August the moisture available to western yellow pines planted in the open had been completely exhausted, whereas a little available moisture still remained under the bushes. This observation led directly to an examination of each of the 1,800 trees in the plantation, since, theoretically at least, the trees in the open should be found dying in large numbers as a result of the more intense drought in the open spaces. The actual condition of the plantation at that time is shown in Table 4.

TABLE 4.—Average condition on August 20 of western yellow pines^a planted April 9-13, 1926, among *Ceanothus* bushes on an east slope in western Montana

Condition	Open position	Intermed- iate	Under <i>Ceano-</i> <i>thus</i>
	Per cent	Per cent	Per cent
Thrifty.....	40	57	80
Unthrifty.....	13	11	9
Alive.....	53	68	89
Dead.....	47	32	11

^a Basis, 600 trees in each of the three positions.

This table indicates clearly that the trees planted closely under the brush survived best during the dry season following spring planting. It seemed that the problem was already more than half solved because the first dry season is always the most critical time in the life of the planted trees. The possibly keener competition in the future between the more deeply penetrating roots of the trees and shrubs is not especially to be feared, because the shock associated with planting will have been outgrown and the tops of the bushes will still provide beneficial shelter from extreme atmospheric conditions. So far as the attainment of high survival is concerned, it seems safe to recommend that yellow pines be planted as close to *Ceanothus* bushes as is practicable without appreciably lowering the quality of planting.

However, this early high survival under brush will be useless if the bushes ultimately "smother out" the trees. It is not always true that those environmental conditions which favor plant life in general favor both survival and growth. Sometimes the same conditions that favor survival retard growth and vice versa. Such an observation was made on western white pine in an earlier study (12), in which trees planted on a steep northwest slope survived best of all the lots tested but grew least rapidly, while the trees on the severe west slope survived least well, but grew most rapidly during the first three years following planting. Similarly in the present study it may be found that the conditions that favored high survival under the bushes may be such as to retard growth of the trees. However, it does not seem likely that *Ceanothus* can permanently suppress these yellow pines when one considers the prevalence of brush fields following destructive fires and the widely accepted idea that all stands of timber represent a more advanced stage in plant succession in timber regions than do the shrub types. Proof of the natural

dominance of the trees or brush in this experiment can be finally obtained only by observation of the trees year after year until they have either died or grown out above the bushes. In the meantime it seemed desirable to gather empirically some evidence of the probable result of this association of brush and trees. About a week was spent in scouting through various brush areas to observe whether or not naturally seeded trees were successful when they happened to start life close to various bushes, especially *Ceanothus*. A special hunt was made for trees that had emerged above the brush or died in the attempt. In several cases, where yellow pines were found emerging above dense brush, the trees were found to stand but a few inches from the root collars of older bushes, a condition indicating that the bushes had a start in the race with trees that grew well within the soil areas the bushes utilized. Although many instances were found of trees successfully emerging after apparently keen competition, it seems significant that only one failure was discovered. No statistical summary of these observations can be presented, but Figures 5 to 8 illustrate special cases which have a bearing on the subject.

Such observations appear to indicate that bushes hinder growth of tree seedlings only temporarily, if at all, and that such hindrance will increase the rotation period in timber growing by a negligible amount.

Ceanothus is one of the few nonleguminous plants that is suspected of harboring bacteria that add nitrogen to the soil in available form. Arzberger (1) and Bottomley (4) report finding root nodules on this genus and the writer has observed them on the roots of the local species. That nitrogen in a form available for the use of plants is lost from the soil as a result of forest fires is often overlooked. Yet available nitrogen is one of the elements of soil fertility that most profoundly affects the growth of trees. If stands of *Ceanothus* fix nitrogen in the soil in appreciable amounts on burned areas, they may increase the rate of tree growth for many years after their protection against loss of trees from drought is no longer needed. Except in fully and evenly stocked young stands of pine, *Ceanothus* bushes frequently live among the trees during their sapling and pole stages or longer, and as long as the shrubs do so persist they may be stimulating the growth of the timber, and thus *Ceanothus* may be an asset even beyond its early beneficial effect in forest regeneration.

SUMMARY

During dry summer weather in western Montana it was found that under *Ceanothus* brush atmospheric evaporation was less, relative humidity greater, soil temperature lower, and soil moisture greater in amount than in adjacent open spaces. As a result of such conditions it was also found that survival of western yellow pines during their first dry season following field planting was much higher under the bushes than in the open. Although future records of growth are needed to show definitely whether or not the trees under the brush can successfully emerge, observations of natural reproduction indicate that brush can not permanently suppress tree growth.



FIGURE 5.—The seedling in front of the white paper is 6 years old, 14 inches high, and stands $5\frac{7}{8}$ inches from the root collar of an 11-year-old bush. Although the dense *Ceanothus* brush is 2 to 3 feet high and completely covers the seedling, the tree grew $3\frac{3}{4}$ inches during its last year (1920). Its thrifty condition indicates that it has a good chance to emerge eventually



FIGURE 6.—A, The western yellow pine seedling, in front of the white paper, is 5 years old and 14 inches tall. The service-berry bush is 8 years old and 11 feet 5 inches tall. Both are in thrifty condition; B, this yellow pine is 8 years old and 4 feet 7 inches tall, and the willow bush is 11 years old and 8 feet 7 inches tall. In spite of the fact that the pine stands in actual contact with the root collar of an older and thrifty bush, the tree grew about 8 inches during each of the last 4 years; C, this western pine is 10 years old, 5 feet 9 inches tall. It stands in contact with the root collar of a 10-year-old alder bush that is 14 feet 4 inches tall. Although the tree is slender, as a result of the dense shade, it is healthy and growing

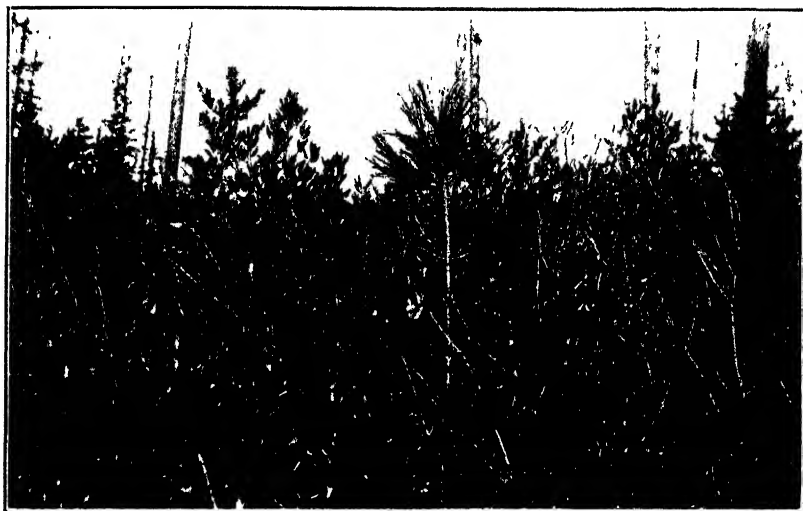


FIGURE 7—The western yellow pine in the center sprang from seed and is now emerging above dense Ceanothus brush. The 11-year-old tree stands 13 inches from the root collar of a 13-year-old bush.



FIGURE 8—The center pine in this picture sprang from seed 13 years ago. It is now well above the 14-year-old Ceanothus bushes that crowd it from all sides. The root collar of the nearest bush is 7 inches from the base of the tree.

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THE NATURE OF SMUT RESISTANCE IN CERTAIN SELFED LINES OF CORN AS INDICATED BY FILTRATION STUDIES¹

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INTRODUCTION

One of the prime considerations in the selection and breeding of crop plants is the development of strains that are resistant to or immune from disease. In general, however, relatively little is known of the fundamental nature of disease resistance in plants. Such is the case in regard to resistance of corn (*Zea mays*) to smut. The corn breeder has produced many lines and crosses resistant to smut and has discovered many facts in regard to the inheritance of resistance and susceptibility in corn,³ yet the fundamental nature of its resistance is not known.⁴ This paper presents the results of experiments to determine whether or not the juices of the various parts of certain resistant strains of corn are factors in resistance and whether or not they contain a substance inimical to the growth of the smut fungus.

MATERIALS AND METHODS

In order to provide plant material of definite genetic constitution and of known smut reaction under field conditions, selfed lines of corn and their crosses were used.

The smut used to furnish inoculum for the filtrates was selected from a group of collections made in various parts of the United States during the summer of 1927 and was cultured from gall tissue. The collection number of each smut and the locality in which it was collected are as follows: No. 3-2, Clemson College, S. C.; No. 34-2, Mesilla Park, N. Mex.; No. 50-1, Davis County Experiment Farm, Utah; No. 60-2, Manhattan, Kans.; No. 74-2, University Farm, St. Paul, Minn.; No. 92-2, Provincetown, Mass.

The corn plants from which the juices were extracted were grown in the greenhouse during the autumn and winter of 1927-28. The experiments were repeated on plants from the same lots of seed grown in the field at the Arlington Experiment Farm, Rosslyn, Va., during the summer of 1928. The plants used were cut and separated into

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² The writer wishes to express his indebtedness and appreciation to the following persons for their cooperation in supplying seed for use in the experiments herein reported: C. H. Kyle, F. D. Richey, J. M. Hamnerly, J. H. Martin, H. N. Vinall, A. M. Brunson, J. R. Hobert, M. T. Jenkins, J. F. Trost, and G. M. Smith, of the Bureau of Plant Industry, U. S. Department of Agriculture; H. K. Hayes and F. R. Immei, of the University of Minnesota; D. F. Jones, of the Connecticut Agricultural Experiment Station; R. J. Garber, of the University of West Virginia; L. E. Melchers and C. H. Ficke, of the Kansas State Agricultural College; A. H. Eddins, of the Iowa State College of Agriculture and Mechanic Arts, and W. T. Conway, of the New Mexico College of Agriculture and Mechanic Arts. The writer is indebted to Mr. Kyle also for many helpful suggestions and criticisms.

³ IMMER, F. R. THE INHERITANCE OF REACTION TO *USTILAGO ZEAE* IN MAIZE. Minn. Agr. Expt. Sta. Tech. Bul. 51, 62 p., illus. 1927.

⁴ Recently Kyle has shown that in certain strains of corn the tightly inclosing husks protect the ears from smut. KYLE, C. H. RELATION OF HUSK COVERING TO SMUT OF CORN EARS. U. S. Dept. Agr. Tech. Bul. 120, 8 p., illus. 1929.

leaf, stalk, and husk parts. These were ground separately through a mill equipped with medium-grade plates. The ground material thus obtained was weighed and the juice extracted for one and one-half minutes by means of a hydraulic press at a pressure of 4,000 pounds. The extracted juice was measured and filtered immediately. The filtration apparatus used in this work has been described in detail in a previous paper.⁵

The apparatus in operation is illustrated in Figure 1.

When sufficient filtrate had been collected in the 250 c. c. Erlenmeyer flasks, the vacuum was released very slowly. The flasks were then disconnected so that each carried the rubber stopper, the bent glass tube filled with sterile cotton, and the short rubber-tube connection (with the screw clamp firmly set) attached to the side arm. The liquid in each flask was thus protected against contamination. The filtrate was removed from the flask by pouring it out through the side arm, after the rubber-tube connection had been removed and the tip of the side arm gently heated.

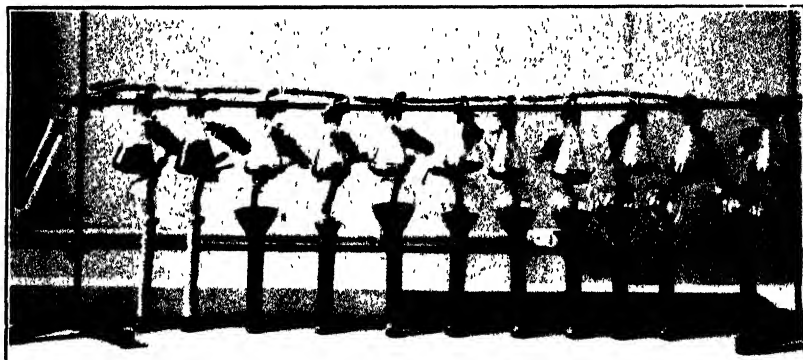


FIGURE 1.—Battery of filtration units in operation. Most of the weight of each unit is supported by the burette clamps around the necks of the Erlenmeyer flasks containing the filtrate. The vacuum is connected by means of glass T tubes and thick-walled rubber tubes of suitable length. All connections not subject to sterilization are shellacked to guard against possible leaks.

After all the flasks of a filtration battery had been disconnected, they were taken into a previously steamed transfer room. Here 5 c. c. portions of the sterile filtrate were measured into sterile test tubes and inoculated with one drop of a smut culture that had grown six days in a nutrient solution.

When the work was first started the filtrates were measured and inoculated by means of sterile pipettes, but this method was extremely slow and resulted in a large number of contaminations. A rapid and satisfactory method involving the apparatus shown in Figures 2 and 3 was worked out. The following technic in using the apparatus was found successful. It was first assembled and sterilized in an autoclave at 15 pounds pressure for 30 minutes. Then the test tube over the end of the burette was removed and the sterile filtrate was poured into the burette from the filter flask through the side arm of the flask after the rubber connection had been removed and the tip of the side arm had been gently heated. The cotton plug in the mouth

⁵ RANKER, E. R. APPARATUS AND METHODS FOR OBTAINING STERILE FILTRATES OF BIOLOGICAL FLUIDS. *Phytopathology* 20: 569-573. 1930.

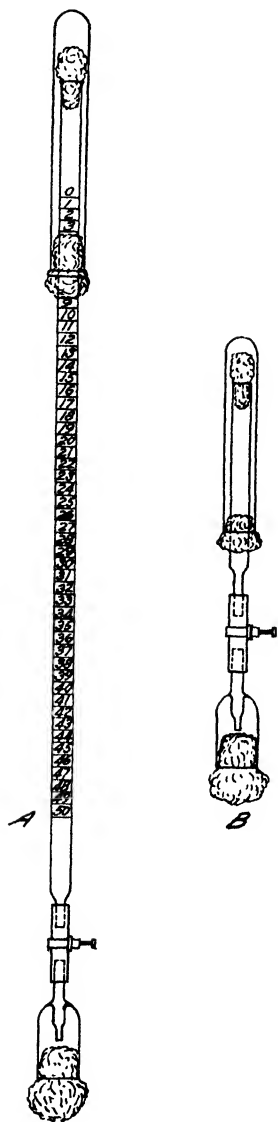


FIGURE 2—Apparatus used in measuring (A) and inoculating (B) sterile plant filtrates: A, Burette for holding a sterile filtrate of extracted juice from corn; B, inoculum tube for holding the liquid medium containing conidia of *Ustilago zeae* in pure culture. The outlet tips with protecting shields are shown just above the lowermost cotton plugs in A and B

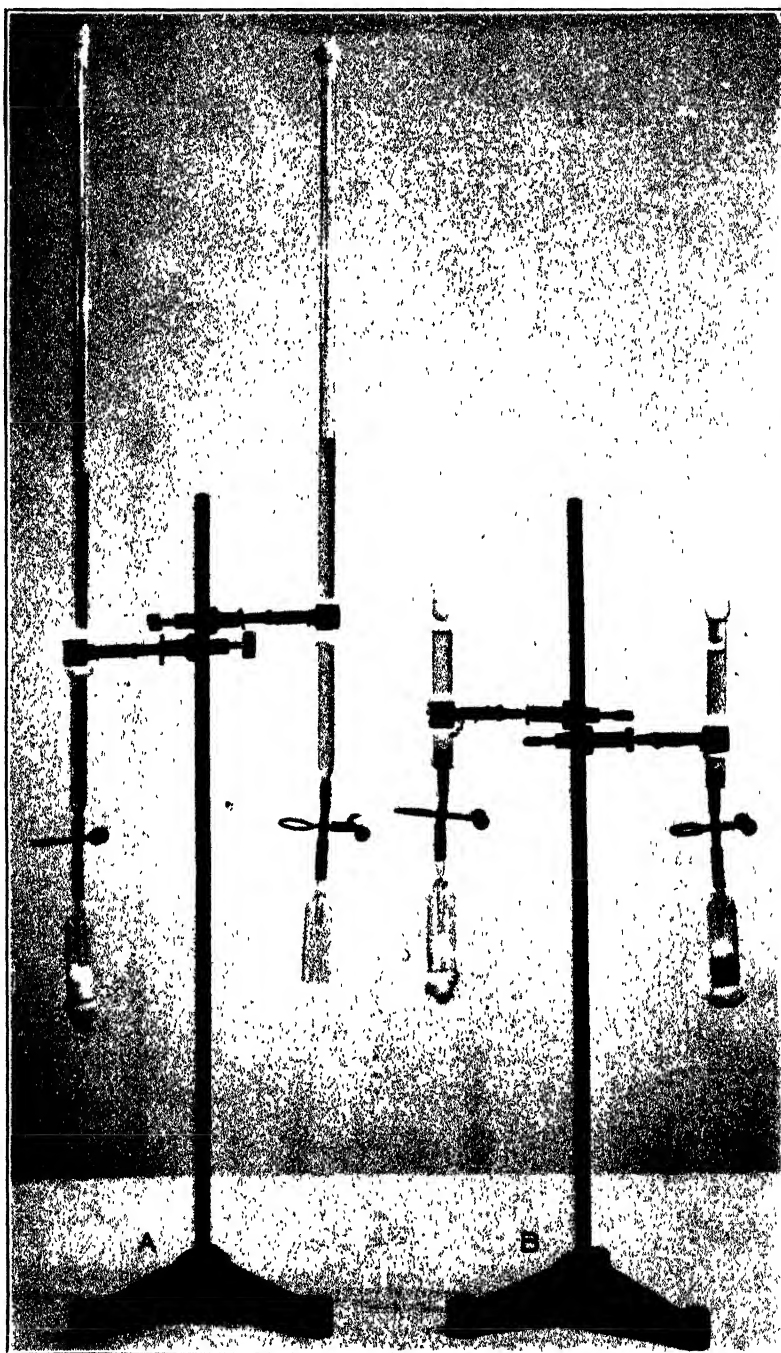


FIGURE 3.—Apparatus shown in Figure 2 set up for operation: A, Burettes containing filtrates; B, tubes containing inoculum. The lower cotton plug has been removed from one of the burettes to show the manner of exposing the outlet tip as described in the text

of the burette was reinserted after the burette was filled. The inoculation device (fig. 2, B) was filled with inoculum in a similar manner. The cotton plug protecting the outlet tip of the inoculation tube was removed and the screw clamp so adjusted that the inoculum would come out at the desired frequency a drop at a time. The sterile test tubes were filled from the burettes with a measured quantity of the sterile filtrate. After each tube was filled it was immediately passed under the inoculation tube to receive one drop of the proper inoculum and then replugged. The cotton plugs protecting the burette tips were not removed until just before the sterile tubes were filled. The glass shields around the outlet tips were large enough to admit the mouth of the test tube readily, but small enough to make it impossible for the test tube to come in contact with the outlet tip. All of the special glass apparatus shown was made from Pyrex glass.

Triplicate cultures of each smut were prepared as above described, and the cultures were allowed to grow at room temperature for a period of one month or six weeks, the period being the same for comparable lots. At the end of the incubation period the amount of growth of the smut was measured in terms of the quantity of fungus obtained by centrifuging at 1,800 revolutions per minute for three minutes. Small conical centrifuge tubes, having standardized calibrations reading to one-tenth of a cubic centimeter, were used.

RESULTS

USING FILTRATES FROM WHOLE CORN PLANTS

Preliminary experiments were undertaken in which filtrates were used that had been obtained by grinding the whole plant excluding the roots. Material representing most of the available smut-resistant and smut-susceptible selfed lines and crosses was tested. Some differences were observed in the quantity of smut developed in the various filtrates, but these were not significant. There was abundant growth of all the smuts in all the filtrates tested. In no case did any of the filtrates show an appreciable inhibiting effect on the growth of *Ustilago zaeae*. This was true regardless of whether the filtrate was obtained from the juice of a resistant or a susceptible selfed line or cross.

USING FILTRATES FROM VARIOUS PARTS OF CORN PLANTS

A second group of experiments was made in which filtrates obtained from various parts of the plant were used. The plants were grown in the greenhouse at the Arlington Experiment Farm during the early autumn of 1927. They were allowed to develop until the silks appeared. At this stage, or during the week following, the plants were harvested and separated into husk, leaf, and stalk parts. The filtrate juices of these parts were used in tests as above described. The results from representative plants are given in Table 1.

TABLE 1.—Growth of *Ustilago zeae* in sterile unheated filtrates of expressed juice from husks, leaves, and stalks of plants of selfed lines and crosses of smut-resistant and smut-susceptible corn grown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., during autumn of 1927

Host material	Degree of resistance *	Part of plant used	Growth of ^b smut culture No —					
			3-2	34-2	50-1	60-2	74-2	92-2
Silver King No. 67...	High.....	Husk.....	0.03	0.07	0.03	0.02	0.05	0.07
		Leaf.....	.15	.20	.15	.10	.10	.15
		Stalk.....	.70	.45	.55	.50	.60	.40
Silver King No. 72...	do.....	Husk.....	.10	.10	.10	.10	.10	.15
		Leaf.....	.25	.30	.25	.20	.25	.30
		Stalk.....	.70	.70	.60	1.00	.70	.70
Rustler No. 35.....	do.....	Husk.....	.05	.05	.06	.05	.07	.20
		Leaf.....	.10	.11	.10	.10	.12	.20
		Stalk.....	.60	.70	.60	.70	.70	.65
Salmon Silk No. 14....	Medium.....	Husk.....	.40	.30	.45	.30	.40	.35
		Leaf.....	.15	.16	.15	.20	.15	.20
		Stalk.....	.90	.90	1.10	1.05	1.05	.80
Kansas Sunflower 3389(3)×3400(7).	Medium to high...	Husk.....	.76	.80	.70	.70	.70	.70
		Leaf.....	.60	.60	.50	.45	.55	.65
		Stalk.....	1.05	1.30	1.25	.90	.55	.60
C. I. 240-F-577e×C. I. 240 F-577c, 1927.....	High.....	Husk.....	.45	.50	.50	.50	.40	.30
		Leaf.....	.45	.30	.45	.50	.40	.40
		Stalk.....	.60	.80	.70	.70	.75	.60
C. I. 207-F-99×C. I. 218-F-69, 1927.....	Low.....	Husk.....	.92	.75
		Leaf.....	.63	.57
		Stalk.....	.45	.50
Brown Aleurone No. 13...	do.....	Husk.....	.30	.37
		Leaf.....	.40	.60
		Stalk.....	.70	.90

* Information furnished by the corn breeders who supplied the seed from which the host materials were grown

^b Growth is expressed as cubic centimeters of fungus thrown down after centrifugation at 1,800 r. p. m. for 3 minutes. Details and reasons for using this method are given in a previous paper: RANKER, E. R. SYNTHETIC NUTRIENT SOLUTIONS FOR CULTURING *USTILAGO ZEA*. Jour Agr. Research 41: 435-443, illus. 1930.

The data of Table 1 show results entirely different from those obtained from the mixed juices of whole plants. There are striking differences in the growth of *Ustilago zeae* in the sterile filtrates of juice from husks, leaves, and stalks. The filtrates of the husk-tissue juice of Silver King No. 67, Silver King No. 72, and Rustler No. 35, varieties highly resistant under field conditions, show a decided inhibiting action on the growth of *U. zeae*. The juice from the leaves of Salmon Silk No. 14, a medium-resistant variety, seems to possess the greatest relative inhibiting action. Juices from two resistant crosses, Kansas Sunflower 3389(3)×3400(7) and C. I. 240-F-577e×C. I. 240-F-577c, seem to possess no marked inhibiting power. Data from two smut-susceptible lines included in Table 1 illustrate a reaction found for all susceptible lines tested, namely, that the filtrate from no plant portion showed any marked inhibiting effect on the growth of *U. zeae*.

It is worthy of note that filtrates inhibiting the growth of *U. zeae* gave the same result with all the smut collections tested, regardless of the locality from which they came, and regardless of the fact that they undoubtedly represented different physiologic forms, two collections (Nos. 60-2 and 74-2) being especially virulent.

DISCUSSION AND CONCLUSIONS

The foregoing results indicate a type of resistance in which the host contains some soluble substance in the tissue juice that inhibits the growth of *Ustilago zeae*. This inhibiting substance was not

found in the juice of any part of any of the susceptible lines tested nor in some of the resistant lines.

In some of the lines the substance was most inhibiting in the husk juice, as in Silver King No. 67. In other lines the leaf juice was relatively most inhibiting to the fungus growth, as in Salmon Silk No. 14. The fact that some smut-resistant lines of corn fail to give positive results from the filtrate studies indicates the existence of other types of resistance. Lines of corn that possess resistance due to some inhibiting substance present in their juices conceivably may be resistant because of other factors also.

One interesting feature of the results obtained in this study was the constancy of the inhibitory effect with all the smut cultures tested, regardless of difference in physiologic forms and virulence. As noted above, two of the smut cultures were especially virulent on most of the pure lines and crosses. This uniform result is significant in view of complications presumably introduced by different physiologic forms of smut.

There is need of more information and study. The data at hand, however, are suggestive of a promising objective in corn breeding. The methods here reported might well serve to aid in selecting selfed lines and crosses possessing resistance to the smut organism.

INFLUENCE OF HULLING THE CARYOPSIS ON COVERED-SMUT INFECTION AND RELATED PHENOMENA IN OATS¹

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INTRODUCTION³

In the course of breeding experiments for the development of economic strains of oats resistant to *Ustilago levis* (K. and S.) Magn., the writers have recorded data on the influence of removing the hull from the caryopsis on (1) covered-smut infection, (2) seedling emergence, (3) number of plants reaching maturity, and (4) inheritance of resistance to covered smut.

Investigators are not in accord regarding the value of hulling before artificially inoculating seed with covered-smut spores for the determination of relative susceptibility and resistance in oats. In the present investigations, in which thousands of plants were grown in comparable experiments from inoculated unhulled and hulled seed, the number of smutted plants was greatly increased by hulling. It is generally held that the spores of the covered-smut fungus are brought in contact with the seed in the threshing of healthy and smutted panicles, and that the smut spores overwinter on the exterior of the hulls. In the process of threshing, a small percentage of the seed usually is hulled. In view of this fact, it is desirable in breeding oats to select hybrids that are resistant to or immune from covered smut even when the inoculum is applied to the naked kernel or caryopsis.

In connection with this study some data were obtained on the percentage of seedling emergence and on the number of plants maturing from unhulled and hulled seed. The influence of hulling on the number of smutted and smut-free segregates in hybrid populations also was noted.

These studies were made at Ames, Iowa; Dickinson, N. Dak.; Moro, Oreg.; Aberdeen, Idaho; and Moccasin, Mont., in cooperation with the respective State agricultural experiment stations. At Ames conditions are typical of the so-called Corn Belt and are entirely distinct from the dry, semiarid conditions prevailing at Dickinson, Moccasin, and Moro. At Aberdeen still another set of conditions prevails—an arid climate, with the water for crop production supplied by irrigation. The value of the data is increased by the wide variation in climatic and other environmental conditions under which they were obtained.

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² The writers are indebted to L. C. Burnett, Chief in Cereal Breeding, Iowa Agricultural Experiment Station, for assistance in conducting the experiments at Ames.

³ In several previous publications of the Office of Cereal Crops and Diseases the term "dehulled" was used to designate the removal of the glumes (lemma and palea) from the caryopsis or groat. In this paper "unhulled" designates the kernel with the glumes attached, and "hulled" the caryopsis from which the lemma and palea have been removed.

REVIEW OF LITERATURE

As early as 1887 Jensen (11)⁴ observed that smut infection in oats was increased by removing the hull before artificially inoculating the seed. Johnston (12) reported that the removal of the glumes (hulls) from oats caused a large increase in the percentage of infection by loose and covered smuts. The increase in infection was largest in susceptible and moderately susceptible varieties and much smaller in resistant varieties. A study was made of the smut reaction of plants grown from hulled seed of the Burt and Kanota varieties, resistant to the inoculum used. The data indicated that Burt derived part of its resistance to smut from the mechanical protection afforded by the glumes. The resistance of Kanota was not primarily due to glume protection. Johnston concludes that some varieties of oats escape infection through the protection of their glumes, while others are relatively free from smut because of inherent resistance.

One hundred per cent infection, however, is not obtained with hulled seed. Gage (6) suggests that some of the covered-smut spores are disseminated in time to enter late-blooming flowers while the glumes are open and that these spores are a more effective source of inoculum than those that are brought in contact with the outside of the hulls in threshing.

Gaines, as reported by Schafer (19, p. 31), and Stanton, Stephens, and Gaines (21) present data which indicate that a much higher infection with *Ustilago levis* was obtained by removing the oat hulls.

Garber, Giddings, and Hoover (9) tried various methods, including hulling seed before treating with smut, in producing an artificial epidemic, but made no direct statement relative to the effect of hulling.

Sampson (18) notes that removing the husk (hull) increased infection of the Potato variety of oats as much as 33.8 per cent, and that of *Avena strigosa pilosa* with *Ustilago avenae* and *U. levis* 42.9 per cent. She also notes that susceptible varieties show considerably increased infection after hulling but that resistant varieties are not affected.

Bayles and Coffman (2) present data which show that a much higher infection of covered smut is obtained by hulling the oat caryopsis.

Tisdale (22) and Tisdale and Tapke (23) have demonstrated that infection of barley with loose and covered smuts can be increased by removing the glumes before applying smut spores to the seed.

Barney (1), Reed (13, 14), Reed, Griffiths, and Briggs (16), Reed and Faris (15), Reed and Stanton (17), Wakabayashi (24), Gaines (7, 8), and Hayes, et al. (10) all have reported experiments on the relative susceptibility or on the inheritance of resistance to smut of oats when the hull was not removed before inoculation.

MATERIALS AND METHODS

Ten varieties, 200 pure-line selections from the unnamed oat C. I.⁵ No. 357, and hundreds of selections from seven different crosses, were used in the studies reported in this paper. The history of this plant material is presented in the following paragraphs. Methods of inoculating seed and of recording data also are outlined briefly.

⁴ Reference is made by number (italic) to Literature Cited, p. 632

⁵ "C. I." indicates accession number of the Office of Cereal Crops and Diseases.

PARENT VARIETIES

The varieties used as parents of hybrids were Markton, Aurora, Idamine, Iogren, Scottish Chief, Silvermine, Swedish Select, and Victory. All of these varieties belong to the common oat group, *Avena sativa*.

MARKTON.—The history, description, and probable value to oat breeding of the smut-immune variety Markton (C. I. No. 2053) have been recorded by Stanton, Stephens, and Gaines (21). It is an early to midseason variety with large panicles and rather long, slender to midplump, yellow kernels. So far as known, Markton has been uniformly immune from the smuts of oats in all experiments conducted in the United States. Markton is of commercial importance in Oregon, Washington, Idaho, and adjacent Montana.

AURORA.—This variety (C. I. No. 831) has not been described previously. It originated at the Arlington Experiment Farm, Rosslyn, Va., as a selection from Red Rustproof. The original selection was made by C. W. Warburton in 1909. Aurora has been grown experimentally from both fall and spring seedling. It is an early, short to midtall variety with a very short, plump, yellow awnless kernel. The variety is not economically important. It is susceptible to both loose and covered smut.

IDAMINE.—Stanton, Griffec, and Etheridge (20) have described Idamine (C. I. No. 1834). It is a midseason variety with very white kernels, and is moderately susceptible to the smuts of oats. Idamine is of some economic importance under irrigation in southern Idaho.

IOGREN. This variety (C. I. No. 2024) has been described by Burnett, Stanton, and Warburton (3) and by Stanton, Griffec, and Etheridge (20). It is a midseason variety with yellow kernels and is of considerable economic importance in northern Iowa. Iogren is highly susceptible to the smuts of oats.

SCOTTISH CHIEF. This variety (C. I. No. 1699) apparently was introduced from Scotland about 30 years ago. Scottish Chief is described by Etheridge (5) as a midseason white oat. Scottish Chief is of minor importance as an agricultural variety in the United States.

SILVERMINE.—Silvermine (C. I. No. 1013) apparently was first introduced to the seed trade in 1897 by a commercial seed company of La Crosse, Wis. It is a standard midseason white variety and is generally grown throughout the northern part of the United States. It is described by Etheridge (5).

SWEDISH SELECT.—This variety (C. I. No. 134) was introduced into this country by the United States Department of Agriculture in 1899 (4). Swedish Select is a midseason white oat of considerable commercial importance in the United States. It has been described by Reed and Stanton (17) and by Etheridge (5).

VICTORY.—This variety (C. I. No. 560) was originated about 1904 at the Plant Breeding Station, Svalof, Sweden. It is a pure line of the old Probstieier variety. It was first introduced into the United States in 1908 by the United States Department of Agriculture. Victory is a midseason white oat and differs mainly from Swedish Select in having fewer awns and a slightly less plump kernel. Owing to its high yield and excellent kernel characters Victory has become a leading variety in the irrigated sections, as well as in the more northern oat sections of the United States.

Reed, Griffiths, and Briggs (16) presented data showing Aurora, Scottish Chief, Silvermine, and Victory as highly susceptible to the smuts of oats. They indicated Swedish Select as moderately susceptible. These results agree fairly well with those presented in this paper. However, in the writers' experiments Scottish Chief seemed to possess more resistance than any of the other varieties crossed with Markton. Swedish Select, on the other hand, was more than moderately susceptible in the writers' tests.

In addition to Markton the Early Champion and Sixty-Day varieties have been used for the study of the influence of hulling on emergence. The origin of Early Champion (C. I. No. 1623) has not been determined. Etheridge (5) described it as an early, white-kernelled variety. Early Champion was grown extensively in the Corn Belt a quarter of a century ago, but to-day it is of little commercial

importance. It is highly susceptible to the smuts of oats, as observed many years ago and confirmed by Reed, Griffiths, and Briggs (16).

The Kherson or Sixty-Day variety has been fully described by Warburton and Stanton (25). Most of the strains of this oat have been moderately resistant or moderately susceptible to the smuts of oats. The particular strain of Sixty-Day (C. I. No. 165-1) used in the experiment herein reported is highly susceptible to the smuts of oats, as shown by Bayles and Coffman (2).

SELECTIONS STUDIED

The smut resistance of the unnamed parent variety C. I. No. 357, from which the smut-immune variety Markton was selected, has been a matter of some interest. This unselected introduction had been discarded on most agricultural experiment stations several years before the discovery of the value of Markton. Through John H. Martin⁶ remnant seed was located at the Belle Fourche Field Station, Newell, S. Dak. An increase plot from this seed was grown at Aberdeen, Idaho, in 1924, from which the selections used in the studies reported herein were made.

CROSSES STUDIED

Studies were made on relative smut infection in progenies grown from unhulled and hulled seed of the following crosses involving the parent varieties discussed previously:

Markton×Scottish Chief
Markton×Idamnine
Markton×Victory
Markton×Swedish Select

Aurora×Markton
Iogren×Markton
Silvermine×Markton

The first five of these crosses were made by G. A. Wiebe at the Aberdeen Substation, Aberdeen, Idaho, in 1923. The last two were made at the same station by T. R. Stanton in 1924. The F_1 plants of the first five crosses were grown in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the winter of 1923-24. The F_2 populations were grown at the Aberdeen Substation in 1924. The F_1 plants of the last two crosses were grown at the Arlington Experiment Farm in the winter of 1924-25, and the F_2 populations at the Aberdeen Substation in 1925.

SEED INOCULATION

The inoculum used in these experiments came from a collection made at the Aberdeen Substation, Aberdeen, Idaho, in 1924. This collection was predominantly *Ustilago levis* with a trace of *U. avenae*. Fully 99 per cent of the spores were entirely smooth (*U. levis*), the remaining 1 per cent being minutely echinulate (*U. avenae*).

One method of applying the inoculum was followed uniformly throughout these experiments. The seed for each row was placed in its respective seed envelope and a liberal quantity of smut spores was added. The envelope was then shaken vigorously to cover the seed thoroughly with the spores.

In 1925 one half of the seed of each selection or variety included in the various smut experiments was hulled, while the other half was not hulled before inoculation. This method also was largely followed

⁶ Of the Office of Cereal Crops and Diseases.

in 1926. Because of the marked increase in the number of smutted plants obtained from hulled seed in 1925 and 1926, all seed sown in these experiments since the latter year was hulled before being artificially inoculated with smut spores. For comparing the relative infection in progenies grown from unhulled and hulled seed data are available only for 1925 and 1926. The hulls were removed in the laboratory with small dissecting tweezers or a penknife blade.

RECORDING OF DATA

The percentage of smut infection was calculated on the basis of the plant as the unit. Any plant showing the merest trace of smut was considered infected.

EXPERIMENTAL DATA

INFLUENCE OF HULLING ON INFECTION IN VARIETIES AND HYBRIDS

Table 1 gives the data on the smut infection of the crop grown from unhulled and hulled seed for the different varieties and their hybrids.

A decidedly higher percentage of smutted plants was obtained in the progenies grown from hulled seed. At Aberdeen in 1925 the average percentage of infection for the three parent varieties grown from hulled seed was 72.6 per cent and from unhulled seed 44.0. In the hybrid progenies the percentages obtained from hulled and unhulled seed were 11.4 and 4.7, respectively. The results at Aberdeen in 1926 varied slightly from those of 1925, but were similar, and for the two years were consistent and conclusive.

At Ames, Iowa, however, a less satisfactory infection was obtained, owing to unfavorable weather conditions at seeding and thereafter. The four parent varieties in 1926 showed an average infection from hulled seed of 50.2 per cent as compared with 16.2 per cent for unhulled seed. In the selections from crosses the percentages were 9.2 and 0.5, respectively.

At Dickinson, N. Dak., in 1925, Swedish Select showed an average infection of 80.6 per cent in the plants grown from hulled seed, while 75.6 per cent of those grown from seed not hulled were infected. The relative difference in the parent varieties between unhulled and hulled seed for some reason is much less at Dickinson than at Aberdeen or Ames. The selections from the Markton × Swedish Select cross at Dickinson, however, showed an average infection of 14.8 per cent from hulled seed as compared with 3.6 per cent from unhulled seed.

The Scottish Chief variety at Moro, Oreg., in 1925 produced 24.6 per cent smutted plants from hulled seed and 11.1 per cent from unhulled seed. A similar spread in infection for the two classes of seed was obtained in the selections of the Markton × Scottish Chief cross.

In all, 2,848 parental and 14,670 hybrid plants were studied. Hulling the seed of the parent varieties increased infection in the resulting crop from 35.2 per cent in plants grown from unhulled seed to 63.8 per cent from hulled seed, and in the hybrids from 3.8 per cent in plants grown from unhulled seed to 12.8 per cent from hulled seed. Where smut-resistant strains of oats are a primary object in the breeding program, greater progress may be made by hulling the seed.

TABLE 1.—*Smut infection* ^a of oat varieties and hybrid populations grown from unhulled and hulled seed at one or more of four experiment stations in 1925 and 1926

Station and year	Parent or hybrid	Plants grown from—									
		Hulled seed					Unhulled seed				
		Total	Number	Infected	Total	Number	Total	Number	Infected	Total	Number
Aberdeen, Idaho: 1925	Idamune	158	121	76.6	302	157	62	30.7	9	5.7	3.7
	F ₁ Markton×Idamune	161	33	20.5	210	119	56.7	100	44.0	8.4	4.7
	Aurora	202	110	54.5	210	2,659	92	52	24.0	3.8	3.8
	F ₂ Markton×Aurora	151	140	92.7	208	618	52	24.0	3.8	3.8	3.8
	Victory	151	140	92.7	208	618	52	24.0	3.8	3.8	3.8
Total or average	F ₂ Markton×Victory	622	117	18.8	621	273	44.0	161	4.7	4.7	4.7
	(Parents)	311	371	72.6	621	3,431	161	4.7	4.7	4.7	4.7
	(Hybrids)	3,430	392	11.4	189	49	25.9	5	39.4	10.1	10.1
	Idamune	170	104	61.2	189	79	41.8	5	25.9	6.3	6.3
	F ₁ Markton×Idamune	87	136	76.8	218	86	39.4	7	33.2	8.1	8.1
Total or average	Victory	177	16	21.3	69	135	12	33.2	8.1	8.1	8.1
	F ₂ Markton×Victory	75	16	21.3	69	135	12	33.2	8.1	8.1	8.1
	(Parents)	347	240	69.2	407	148	33.2	12	8.1	8.1	8.1
	(Hybrids)	162	28	17.3	146	32	21.9	0	9.1	0	0
	Idamune	110	68	61.8	146	680	7	0	9.1	0	0
Anes, Iowa, 1926	F ₁ Markton×Idamune	72	29	40.3	77	802	2	4.7	1.7	1.7	1.7
	Victory	41	16	39.0	43	360	10	20.4	4.5	4.5	4.5
	F ₂ Markton×Victory	300	70	23.3	49	67	51	16.2	5.5	5.5	5.5
	Logen	44	21	47.7	49	67	51	16.2	5.5	5.5	5.5
	Silvermine	56	12	21.4	315	1,809	9	75.6	3.6	3.6	3.6
Total or average	F ₂ Markton×Silvermine	267	114	50.2	9.2	82	1,833	66	11.1	10.4	10.4
	(Parents)	960	88	9.2	82	1,833	66	11.1	10.4	10.4	10.4
	(Hybrids)	103	83	80.6	82	1,833	66	11.1	10.4	10.4	10.4
	Swedish Select	114	28	24.6	81	393	41	35.2	3.8	3.8	3.8
	Scottish Chief	585	114	19.5	1,506	7,017	289	3.8	3.8	3.8	3.8
Total or average	F ₂ Markton×Scottish Chief	1,342	886	63.8	12.8	1,506	7,017	289	3.8	3.8	3.8
	(All parents)	7,063	906	12.8	1,506	7,017	289	3.8	3.8	3.8	3.8
	(All hybrids)	1,342	886	63.8	12.8	1,506	7,017	289	3.8	3.8	3.8
	Swedish Select	103	83	80.6	82	1,833	66	11.1	10.4	10.4	10.4
	Scottish Chief	114	28	24.6	81	393	41	35.2	3.8	3.8	3.8
Total or average	F ₂ Markton×Scottish Chief	1,342	886	63.8	12.8	1,506	7,017	289	3.8	3.8	3.8
	(All parents)	7,063	906	12.8	1,506	7,017	289	3.8	3.8	3.8	3.8
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	Swedish Select	103	83	80.6	82	1,833	66	11.1	10.4	10.4	10.4
	Scottish Chief	114	28	24.6	81	393	41	35.2	3.8	3.8	3.8

^a With *Ustilago lentis* (K. and S.) Magn^b No smut was observed in any lines. Owing to unfavorable weather no infection occurred^c No data are presented for Markton, as this variety produced no smut in either unhulled or hulled seed at any station in any year.

INFLUENCE OF HULLING ON INFECTION IN SELECTIONS FROM THE UNNAMED
OAT C. I. NO. 357

Results were obtained also on relative smut infection occurring in selections of the unnamed oat C. I. No. 357, grown from unhulled and hulled seed. In all, 200 of these selections were grown in the experiment. Owing to lack of time, the seeds of only 100 selections were hulled for the 1925 crop. An equal number of seeds per row was sown in both the unhulled and hulled groups. Table 2 gives the total number of plants and the number of infected plants from unhulled and hulled seeds.

TABLE 2.—*Smut infection in plants grown from equal numbers of unhulled and hulled seeds of 200 selections made from the unnamed oat C. I. No. 357*

Condition of seed	Plants		
	Total		Infected
	Number	Number	Per cent
Hulled.....	1,412	236	16.7
Unhulled.....	1,747	41	2.5

Hulling markedly reduced the number of plants that reached maturity, and increased the amount of infection. Only 2.5 per cent of the plants grown from unhulled seed were smutted, while 16.7 per cent of the plants grown from hulled seed were infected.

The influence of hulling the seed for the 1925 crop was evident in the 1926 progeny. Of 112 selections which showed no infection in 1925, 44 showed infection in the 1926 test when all seed was hulled. Of these 44 lines, 12 were grown from hulled seed and 32 from unhulled seed in 1925. Table 3 gives a summary of the 1926 data showing 1925 seed treatment.

TABLE 3.—*Smut infection* in the two plant groups resulting from 1925 seed treatment of 44 selections of the unnamed oat C. I. No. 357*

Selections (number)	Condition of seed	Plants grown at—					
		Aberdeen, Idaho			Moro, Oreg		
		Total	Infected		Total	Infected	
		Number	Number	Per cent	Number	Number	Per cent
12.....	Hulled.....	441	14	3.2	313	3	1.0
32.....	Unhulled.....	1,012	80	7.7	780	43	5.5
Total or average		1,483	94	6.3	1,093	46	4.2

* With *Ustilago levis* (K. and S.) Magn.

The progenies of plants grown from seed that was not hulled the previous year showed more than three times as much infection in 1926 as the progenies originating from a hulled-seed source. This is conclusive evidence of the value of hulling for determining true smut susceptibility in oats.

Under the conditions of these experiments consistently higher infection was obtained by hulling the oat kernel. All of the parent

varieties other than Markton, with the possible exception of Scottish Chief, are moderately to highly susceptible to the smuts of oats. Even in these susceptible varieties, however, the glumes apparently offer considerable protection against infection.

The data for selections from the unnamed oat C. I. No. 357 emphasize the fact that susceptible strains can be eliminated more certainly if seed is hulled. Under field conditions a 100 per cent infection is rarely obtained even where hulling is practiced, but the number of escapes is fewer than when the hulls are not removed.

The data from these experiments are of interest in considering also the susceptibility of the naked oat (*Avena nuda*) to covered-smut infection. There is no reason to believe that *A. nuda* is inherently more susceptible than many varieties of *A. sativa*. Lacking the protection of hulls, it appears to be more highly susceptible. Resistance in the naked oat must be developed by crossing with varieties like Markton, which are truly resistant and are not smut-free merely because of hull protection.

INFLUENCE OF HULLING ON SEEDLING EMERGENCE

Limited data are available from the experiments conducted at Moro, Oreg., in 1926, on the influence of hulling on seedling emergence. The data are given in Table 4.

TABLE 4.—Influence of hulling oat seeds on seedling emergence at Moro, Oreg., in 1926

Variety	Seeds of each condition sown	Seedlings from uninoculated seed					
		Unhulled		Hulled		Reduction due to hulling	
		Number	Per cent	Number	Per cent	Number	Per cent
Early Champion	760	602	77.9	511	67.2	81	10.7
Sixty-Day	760	572	75.3	540	71.1	32	4.2
Markton	680	532	78.2	503	74.0	29	4.3
Total or average	2,200	1,696	77.1	1,554	70.6	142	6.5

Variety	Seeds of each condition sown	Seedlings from seed inoculated with <i>Ustilago levis</i>					
		Unhulled		Hulled		Reduction due to hulling	
		Number	Per cent	Number	Per cent	Number	Per cent
Early Champion	760	602	79.2	478	62.9	124	16.3
Sixty-Day	760	554	72.9	481	63.3	73	9.6
Markton	680	514	75.6	465	68.4	49	7.2
Total or average	2,200	1,670	75.9	1,424	64.7	246	11.2

Hulling reduced the number of seedlings emerging from uninoculated seed of all varieties 6.5 per cent and from seed inoculated with *Ustilago levis* 11.2 per cent. Smut inoculation influenced the emergence of seedlings, but hulling alone reduced the number of seedlings even slightly more than did the smut. The effect of hulling varied considerably with the variety. Early Champion, which produces a rather small caryopsis and is not especially vigorous, suffered an average reduction of 13.5 per cent, which was greater than that of any other variety. Hulling reduced by an average of 6.9 per cent

the emergence of seedlings in Sixty-Day, another variety with a small caryopsis but of greater vigor than Early Champion. Markton was affected less both by hulling and by inoculation than either of the other varieties. It seems rather significant, however, that even in Markton, which so far has shown no apparent evidence of smut infection, the seedling emergence was reduced by 2.9 per cent through inoculation with smut. If this may be accepted as evidence, it shows that Markton is not completely free from infection by the smut organism.

INFLUENCE OF HULLING ON NUMBER OF PLANTS REACHING MATURITY

In nearly all experiments in the present investigations in which hulled seed was compared with unhulled seed, a record was made of the number of seeds sown. As a result, some data were obtained on the influence of hulling on the number of plants that reached maturity. Where emergence counts are not available a comparison of the number of plants maturing from equal numbers of hulled and unhulled seed is of interest.

Data are available on this phase of the investigations from essentially the same varieties and hybrids used in the studies on the influence of hulling on smut infection. Likewise, data are available only for 1925 and 1926. These have been summarized and are shown in Table 5.

TABLE 5.—*Influence of hulling oat seeds on the number of plants reaching maturity*

Station and year	Gen-eration of hybrid progeny	Variety crossed on Markton	Parents* or hybrids	Plants from—					
				Hulled seed			Unhulled seed		
				Seeds sown	Matured plants		Seeds sown	Matured plants	
				Number	Number	Per cent	Number	Number	Per cent
Aberdeen, Idaho 1925	F ₁	Aurora	Parents	750	511	68.1	750	621	82.8
		Idamne	Hybrids	10,150	7,935	78.2	10,150	8,254	81.1
	F ₁	Idamne	Parents	600	412	68.7	600	490	81.7
		Victory	Hybrids	2,700	1,916	70.7	2,700	2,150	79.6
Ames, Iowa, 1926	F ₂	Idamne	Parents	850	297	34.9	850	361	42.5
		Silvermine	Hybrids	8,200	2,962	36.1	8,200	3,547	43.3
	F ₂	Swedish Select	Parent	250	103	41.2	238	82	34.5
		Hybrid	Hybrid	6,200	2,722	43.9	5,989	3,008	50.2
Dickinson, N. Dak., 1925	F ₂	Scottish Chief	Parent	160	98	61.3	160	81	50.6
		Hybrid	Hybrid	3,280	1,183	36.1	3,280	1,778	54.2
	Total or average		Parents	2,610	1,421	54.4	2,598	1,635	62.9
			Hybrids	30,530	16,712	54.7	30,319	18,717	61.7

* Data on Markton omitted from table

In general, the data of Table 5 are consistent in indicating that hulling reduced the number of plants that reached maturity. Doubtless most of this reduction is traceable to failure of germination or emergence of seedlings.

At Aberdeen in 1925 the average percentage of plants maturing from hulled seed of the parent varieties and of hybrid selections was 68.1 and 78.2, respectively. For the unhulled seed the percentage was 82.8 and 81.1, respectively. More consistent results were ob-

tained at Aberdeen in 1926. In the two years at this station hulling reduced the number of plants reaching maturity on an average by 13.9 per cent in the parents and 4.2 per cent in the hybrid strains.

Results from Ames are available only for 1926. Owing in part to unfavorable weather conditions for germination and emergence, less than half the seed sown produced matured plants. However, the ratio in reduction caused by hulling is about the same in the parent varieties as in the hybrid strains, the average reduction being 7.6 and 7.2 per cent, respectively.

At Dickinson, N. Dak., and Moro, Oreg., also, a much smaller number of plants reached maturity from both hulled and unhulled seed. The data on Swedish Select and Scottish Chief, the parent varieties, show that more plants reached maturity from hulled than from unhulled seed. This is contrary to the results obtained from the hybrid strains at the same stations as well as to those from both parents and hybrids at all other stations. Soil inequalities and the small number of seeds sown probably account for this variation.

The data obtained at Dickinson on the hybrid selections are in conformity with those obtained at Ames and Aberdeen. Hulling the Markton \times Swedish Select strains reduced the number of plants maturing by 6.3 per cent. In strains of the Markton \times Scottish Chief cross at Moro, hulling reduced plant numbers by 18.1 per cent. This reduction is nearly three times that occurring at any other station. Unfavorable soil conditions at seeding time at Moro probably account for this wide departure.

The results indicate the value of the oat hull (lemma) as a protection to the caryopsis. They probably explain the low germination and rapid loss of germinating ability in the naked or hull-less oat.

In most cases a higher percentage of plants from hulled seed reached maturity from hybrid seed than from susceptible parent seed. The greater survival probably may be attributed to the greater vigor of the hybrids.

INFLUENCE OF HULLING ON CLASSIFICATION OF SEGREGATES IN STUDYING INHERITANCE OF SMUT SUSCEPTIBILITY AND RESISTANCE

Other writers have shown that hulling oats increases infection by smut. A comparison of the infection of hybrid segregates in populations grown from unhulled and hulled seed has not been reported heretofore. Some data of this nature are given in Table 6. The seeds from each F_2 plant were divided into two duplicate lots. The seeds of one lot were then hulled and those of the other were left unhulled. All seeds were inoculated. The seeds from each lot from each F_2 plant were sown in separate rows; that is, each row in each lot duplicated a comparable row in the other lot. The F_3 progenies were classified into four groups: (1) Those infected in the rows from only the hulled lot; (2) those infected in the rows from only the unhulled lot; (3) those infected in the comparable rows from both the hulled and unhulled lots; (4) and those not infected.

TABLE 6.—*Influence of hulling oat seeds on infection of F₃ hybrid progenies with Ustilago levis in 1925*

Variety crossed on Markton	Station	Number of progenies				Total
		Infected, from—			Not infected	
		Hulled seed only	Unhulled seed only	Seed in both lots		
Swedish Select.	Dickinson, N. Dak.	42	4	28	50	124
Idamune.....	Aberdeen, Idaho..	1	0	3	22	26
Victory.....	Aberdeen, Idaho..	4	3	10	19	36
Aurora.....	Aberdeen, Idaho..	19	6	42	74	141
Scottish Chief..	Moro, Oreg.....	30	4	14	17	95
Total....		96	17	97	212	422

The proportions of infected populations from hulled and unhulled seed are very marked. Of the 422 F₃ progenies there were 96 infected only when the seed was hulled, 17 infected only when the seed was not hulled, 97 infected when the seeds were in comparable rows from both the hulled and unhulled lots, and 212 not infected at all. Assuming that the 96 lines infected only when the seed was hulled would have escaped infection in an experiment involving only unhulled seed, under such circumstances there would have been 308 smut-free and 114 infected progenies instead of the 212 smut-free and 210 infected actually obtained. The results would have compared favorably with those obtained by investigators from experiments in which hulling the seed before inoculation was not practiced.

Published observations record a decidedly larger percentage of smut-free progenies than of smut-infected progenies in crosses of resistant and susceptible varieties. The results here presented, derived through using hulled seed, indicate that previous data are not conclusive. Later tests in which hulled seed was used showed that additional strains apparently smut free in the F₃ generation were actually smut susceptible in later generations.

The increase in the number of infected strains apparently due to hulling the seed amounted to 22.8 per cent, a sufficient warrant for hulling the seed in conducting genetic studies on smut resistance under field conditions.

The data also emphasize the importance of developing inoculation methods for insuring complete infection. Until this is done it will not be possible to interpret satisfactorily studies on the inheritance of smut resistance or susceptibility.

SUMMARY

Removing the hull from the oat caryopsis before inoculation increased the percentage of smutted plants in susceptible varieties from 35.2 to 63.8 per cent.

In hybrid selections of these varieties with the smut-immune Markton oat, hulling increased the number of plants infected with *Ustilago levis* from 3.8 to 12.8 per cent.

In selections from the unnamed oat C. I. No. 357 hulling increased infection with *Ustilago levis* from 2.5 to 16.7 per cent. Of 112 lines showing no infection in 1925, 44 were infected with *U. levis* in a second

test when all seed was hulled. Of the 44 lines, 12 were grown from hulled seed and 32 were grown from unhulled seed in 1925.

In three varieties hulling reduced by 8.8 per cent the number of seedlings emerging from both inoculated and uninoculated seed. The influence of hulling on seedling emergence apparently varies with the variety.

The average percentage of plants reaching maturity in all tests was 54.7 for hulled and 61.8 for unhulled seed.

Hulling the seed in hybrid populations resulted in a marked increase in the number of smutted plants above that obtained when the seed was not hulled.

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A STUDY OF THE RELATION BETWEEN ACTUAL AND NORMAL YIELDS OF IMMATURE DOUGLAS FIR FORESTS¹

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INTRODUCTION

For the several methods of predicting the yields of even-aged forests that for years have been much discussed, the yield of normal or fully stocked forests has been generally accepted as forming the most desirable basis. For this purpose normal-yield tables are worked up showing the volume of wood produced by a fully stocked forest on any growing site at any age of its life. A fully stocked forest, at least as defined in the Douglas fir region, is one in which the canopy is fairly complete and average maximum cubic-foot volumes are obtained. The growing space, in other words, is well utilized and the volumes are as large as can be expected on the average in unthinned stands.

The first difficulty that comes up when a forest manager or a landowner wishes to apply normal-yield tables to a particular piece of land for the purpose of predicting the volume at some future period is that the actual, or "empirical," stand is not in a normal condition throughout, and therefore normal-yield tables can not be used without a reduction of their values. He may say that since the values must be reduced the yield tables should be based on average, not normal, conditions. A very little observation will lead anyone to conclude that even the actual condition is far from constant. One tract may be heavily stocked, another lightly stocked, or a single tract may have several degrees of stocking. No empirical table would be apt to fit actual cases without appropriate allowances.

Since many forests of Douglas fir have approximately complete canopies, no trouble would be experienced in locating small fully stocked or normal sample plots scattered throughout almost every tract, but over larger areas there are always a number of irregularities. A fire may have gone through, killing a few trees and causing small openings; a windstorm may have broken tops or uprooted trees; insects may have killed small patches here and there; creeks and low wet places may cause breaks in the canopy; steep slopes and precipices may interrupt the stand; or reproduction may not have been successful from the very beginning. In other

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words, a variety of factors, accidental, physiographical, and physiological, contribute to the departure from a normal condition. The usefulness of a normal table as a standard of reference, representing, so to speak, 100 per cent conditions, becomes more and more striking the further one considers the question.

If this concept of normal-yield tables is adopted, such significant questions as the following must be answered:

What is the relation between the actual and the normal forest?

Does this relation change during the life of the stand?

What causes and what constitutes understocking or overstocking?

How is degree of stocking recognized?

What methods of procedure are best when normal tables are to be used to make yield predictions of a forest?

The American literature on the application of normal-yield tables is not complete. Certain rules for judging stocking and predicting growth, based chiefly on European methods, have been followed and are stated in most standard books on forest mensuration and regulation. (Graves, Chapman, Nisbet, Roth, Woolsey.) But no literature can be found covering adequately the relation between the actual stand and the normal stand, nor any investigations which test the soundness of the methods of application that are advocated. Some light upon this phase of the subject may be obtained from studies recently pursued in the Douglas fir region by the Pacific Northwest Forest Experiment Station.

In 1925 a study of normal yields of second-growth Douglas fir (*Pseudotsuga taxifolia*) was completed in Oregon and Washington for the region lying west of the Cascade Range.² During 1926 and 1927, after the normal yield tables for Douglas fir had been completed, a further field study in understocked and overstocked stands was made. This work attempted to answer questions regarding the application of normal tables to actual stands and the relation between normal and empirical stands. It is with this second study that the present paper is concerned.

As used in these Douglas fir yield studies the term "normal" forest, defined as representing a fully stocked condition, allows a certain flexibility in density in regard to both number of trees and volume. In the construction of yield tables this variation is taken into account by allowing a certain range in the basic data. Averages from these ranges are referred to as the 100 per cent, or normal, yields, and all other yields are then expressed in terms of this standard. "Actual" forests are defined as stands actually found over large areas. "Understocked" is the term usually applied to a stand that is poorer than normal, and "overstocked" to one in better than normal condition. These words, however, are not precise, since, as already stated, normal plots in themselves cover a rather wide range of stocking.

The material gathered in the course of the study is divided into three classes. The first class consists of the records taken on 5,014 chains of strip survey, 785 of which were made by R. E. McArdle

² MCARDLE, R. E. THE YIELD OF DOUGLAS FIR IN THE PACIFIC NORTHWEST. Tech. Bul. 201. 56 p., illus. 1930. MCARDLE, R. E. RATE OF GROWTH OF DOUGLAS FIR FORESTS. STANDARD YIELD TABLES FOR FULLY STOCKED SECOND-GROWTH STANDS. West Coast Lumberman 54: 90-95, illus. 1928.

in 1924 and 1925 and the rest by the writer in 1926 and 1927. The second class consists of 493 sample plots selected from the basic data of the normal-yield study, of which 433 were used to cover the complete range of age and site conditions and 118 plots of the 70-year age class alone were used to analyze the effect of eliminating the variable of age. The third class of material consists of an actual yield survey covering 4,190 acres, of which 2,631 acres are classed as being in the immature Douglas fir type. Very little will be said of this last phase of the study, since it pertains to questions outside the scope of this paper.

The 5,014 chains of strip survey were located in 83 different representative second-growth Douglas fir forests. The strips were practically all 1 chain (66 feet) wide; in a few very dense stands the width was reduced to 0.5 chain. Every tree on the strip was calipered, and a constant check was kept upon the age by taking increment borings to the centers of average-sized dominant trees. The average height of the dominant and codominant trees was used as an index of site quality. When differences in age or site class were found, new tally sheets were started. The sheets were also changed after every 10 chains or full-acre tally. The width of the strip was checked by pacing out 33 feet on each side of the tape. A small range finder set to 33 feet probably would be quite satisfactory for this purpose and would show at a glance from the position of the images whether a tree should be included or not. Continuous notes were kept concerning the slope, the character of the stocking, the size, and probable cause of gaps in the Douglas fir canopy.

In the second season's work only the trees of the main canopy were tallied, the understory trees and the hardwoods being left out altogether. In the first season the dead trees were counted and tallied in two classes—old and recently dead—in an attempt to find out whether an advance to a normal condition was indicated by the rate of mortality. The underlying idea was that if an understocked stand were approaching a normal condition, the loss by mortality would be less than in a normal forest. This idea was put to test immediately after the first field season and found to be of no value. Therefore, the tally of the dead trees was dropped in the succeeding season.

The second class of material consists of detailed records taken on temporary sample plots, all rectangular in shape, varying from 0.25 to 1 acre in size. In the normal-yield study the plots were combined by the tracts in which they were taken, and a composite acre was computed for each tract. By this procedure many of the minor variations from acre to acre were disguised. Recomputation of the single plots was deemed necessary for an accurate comparison of the degree of stocking on strips compared to the degree of stocking on picked acres or plots.³

³ The details of the temporary sample plot measurements and of their combination into a normal yield table are described in full by McArdle (See footnote 2)

RELATION BETWEEN ACTUAL STANDS AND NORMAL STANDS

COMPUTATIONS

Mathematical computations are required to show the extent of the difference between the actual forest and the normal stand—how wide a range of stocking may be expected, how large a factor is the presence of small holes in the canopy, and what relation the stand values bear to one another. For this purpose a number of the processes known to graphical and statistical analysis⁴ were applied to the first two classes of material. Only the more important relations appear in this text, since many of the computations failed to be significant.

The data are treated both as individual strip acres and as tract averages. To obtain a measure which would put all sites and ages

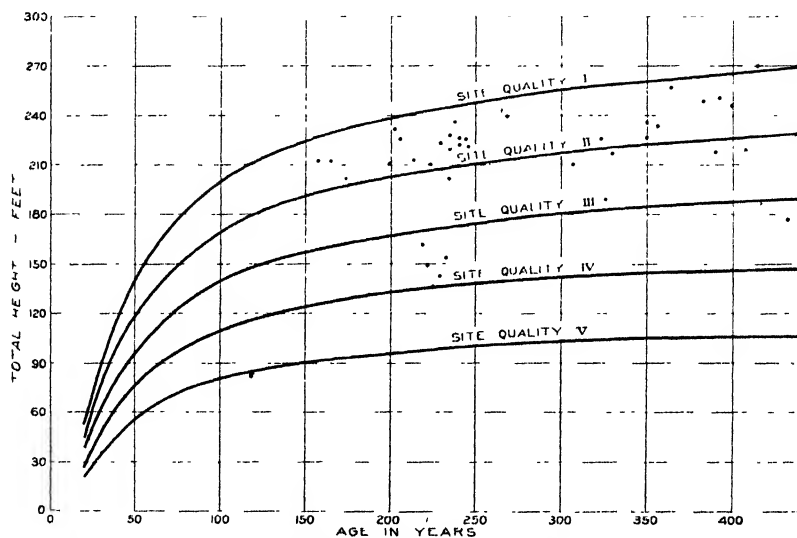


FIGURE 1. Average heights of dominant and codominant Douglas firs to be used in classifying lands by quality of site

on a comparable basis, all the values had to be expressed in terms, or rather percentages, of the normal-yield table values, called variously "degrees of stocking," "normality percentages," "stocking percentages," and so forth. For instance, if a tally shows a total of 260 trees to the acre and a total basal area of 255 square feet on a site and at an age for which the normal values are 300 trees and 280 square feet, the number-of-trees normality percentage is $260 \div 300$, or 87 per cent, and the basal-area normality percentage is $255 \div 280$, or 91 per cent. Similar normality percentages were obtained for (1)

⁴ BRUCE, D., and REINEKE, L. H. CORRELATION ALINEMENT CHARTS IN FOREST RESEARCH. A METHOD OF SOLVING PROBLEMS IN CURVILINEAR MULTIPLE CORRELATION. U. S. Dept. Agr. Tech. Bul. 210, 1930. [In press.]

SMITH, B. B. THE USE OF PUNCH AND TABULATING EQUIPMENT IN MULTIPLE CORRELATION PROBLEMS. U. S. Dept. Agr., Bur. Agr. Econ. 1923. [Multigraphed.]

SMITH, B. B. CORRELATION THEORY AND METHOD APPLIED TO AGRICULTURAL RESEARCH. U. S. Dept. Agr., Bur. Agr. Econ. 1926. [Multigraphed.]

number of trees 11.6 inches d. b. h.⁵ (12-inch class)⁶ and larger, (2) total cubic-foot volume, (3) board-foot volume by International rule with $\frac{1}{8}$ -inch kerf, and (4) board-foot volume by Scribner rule, as well as for (5) the total number of trees and (6) total basal area.

TABLE 1.--Normal yield tables for Douglas fir^a

SITE QUALITY I

Total age (years)	Stand		Total basal area ^b	Average d b h		Average height dominant and co- dominant trees	Volume ^c		
	All trees	12 inches d b h and over		All trees	12 inches d b h and over		Total stand	International rule	Scribner rule
	Number	Number	Sq feet	Inches	Inches	Feet	Cubic feet	Board feet	Board feet
20	571	7	161	5.7	12.6	52	1,830	4,900	400
30	350	57	154	9.0	13.7	92	4,750	24,400	8,000
40	240	109	195	12.2	15.2	120	7,500	47,000	24,400
50	176	126	224	15.3	17.1	139	10,150	70,500	44,100
60	138	118	248	18.2	19.2	156	12,500	90,800	62,000
70	113	105	268	20.9	21.4	170	14,500	108,500	78,200
80	97	93	285	23.3	23.6	181	16,350	124,700	92,500
90	84	84	299	25.6	25.6	192	17,880	137,700	104,800
100	75	75	312	27.6	27.6	200	19,140	148,900	115,100
110	69	69	323	29.4	29.4	207	20,200	157,900	123,700
120	63	63	332	31.1	31.1	213	21,090	165,500	131,100
130	59	59	341	32.7	32.7	218	21,840	172,000	137,700
140	55	55	350	34.3	34.3	221	22,520	178,000	143,500
150	51	51	357	35.8	35.8	224	23,170	183,300	148,700
160	48	48	364	37.2	37.2	226	23,780	188,100	153,500

SITE QUALITY II

20	880		98	4.5		44	1,550	2,100	
30	555	27	150	7.0	13.0	78	4,110	16,000	2,600
40	385	75	189	9.4	13.9	102	6,550	34,900	11,900
50	290	120	217	11.8	15.0	119	8,840	55,000	27,400
60	228	141	241	14.0	16.2	132	10,860	72,800	42,800
70	186	140	260	16.0	17.7	144	12,660	89,000	57,200
80	159	133	276	17.9	19.0	154	14,220	103,200	70,000
90	138	124	290	19.6	20.4	163	15,540	114,700	81,000
100	123	115	302	21.2	21.8	170	16,610	124,400	90,400
110	111	106	313	22.6	23.1	176	17,560	133,000	98,300
120	101	99	322	24.0	24.2	181	18,340	140,300	105,100
130	94	93	331	25.3	25.4	185	19,000	146,500	111,000
140	88	87	338	26.5	26.6	188	19,590	152,000	116,300
150	82	82	346	27.7	27.8	190	20,130	156,700	121,200
160	78	78	353	28.9	28.9	192	20,650	161,100	125,700

SITE QUALITY III

20	1,460		92	3.4		37	1,250	200	
30	865	6	140	5.5	12.5	64	3,300	8,400	300
40	585	37	177	7.4	13.1	84	5,250	22,000	4,500
50	430	79	204	9.3	13.9	98	7,050	37,100	12,400
60	337	118	226	11.1	14.7	109	8,700	52,000	23,800
70	274	139	244	12.8	15.6	119	10,150	65,100	35,200
80	232	148	259	14.3	16.5	127	11,350	77,200	45,700
90	204	149	272	15.6	17.4	134	12,390	86,700	55,000
100	184	145	283	16.9	18.4	140	13,270	94,700	62,800
110	166	139	292	18.0	19.2	145	14,000	101,500	69,400
120	152	134	301	19.1	20.0	149	14,600	107,200	75,000
130	141	128	309	20.1	20.8	152	15,140	112,200	80,000
140	131	123	317	21.1	21.6	154	15,610	116,900	84,500
150	123	117	324	22.0	22.4	156	16,080	121,100	88,600
160	117	113	331	22.8	23.1	158	16,490	125,000	92,400

^a Adapted from tables by R. E. McArdle. (See footnote 2.)

^b Total basal area includes all trees of the stand.

^c Cubic volume includes the total cubic-foot volume inside bark of all the trees in the stand. Board-foot volume by international rule for $\frac{1}{8}$ -inch kerf includes the volume of all trees in the 7-inch diameter class and larger (actually 6.6 inches plus) to a 5-inch top inside bark. Board-foot volume by Scribner rule includes the volume of all trees in the 12-inch class and larger (actually 11.6 inches plus) to an 8-inch top inside bark.

^d D. b. h. = diameter at breast height, or 4.5 feet above average ground level.

TABLE 1.—Normal yield tables for Douglas fir—Continued
SITE QUALITY IV

Total age (years)	Stand		Total basal area	Average d. b. h.		Average height dominant and co- dominant trees	Volume		
	All trees	12 inches d. b. h. and over		All trees	12 inches d. b. h. and over		Total stand	Interna- tional rule	Scribner rule
	Number	Number	Sq. feet	Inches	Inches	Feet	Cubic feet	Board feet	Board feet
20	3,069		81	2 2		29	870		
30	1,472		122	3 9		50	2,270	2,400	
40	927	7	153	5 5	12 6	66	3,560	9,200	200
50	659	29	177	7 0	13 1	77	4,780	19,000	3,300
60	500	58	195	8 5	13 6	86	5,880	28,900	8,100
70	405	90	211	9 8	11 1	94	6,830	37,900	14,000
80	345	114	224	10 9	14 6	100	7,690	45,700	20,100
90	304	130	235	11 9	15 1	105	8,400	52,200	26,000
100	271	141	245	12 8	15 7	110	9,000	58,100	31,400
110	247	146	254	13 7	16 2	114	9,500	63,200	36,300
120	224	149	261	14 6	16 7	117	9,920	67,500	40,700
130	209	150	268	15 3	17 2	119	10,290	71,000	44,700
140	195	149	275	16 0	17 7	121	10,620	74,300	48,300
150	184	147	281	16 7	18 1	123	10,920	77,500	51,600
160	175	144	287	17 4	18 6	124	11,200	80,100	54,600

SITE QUALITY V

20	6,920		64	1 3		21	520		
30	2,700		96	2 6		37	1,330		
40	1,530		121	3 8		48	2,110	1,500	
50	1,050	1	140	4 9	12 4	56	2,840	5,900	30
60	780	12	154	6 0	12 7	63	3,500	10,500	1,100
70	625	27	166	7 0	13 0	68	4,090	15,400	2,400
80	525	44	177	7 9	13 3	73	4,580	20,300	4,400
90	451	62	185	8 7	13 6	77	5,000	24,900	6,900
100	403	78	193	9 4	13 9	80	5,350	28,800	9,600
110	362	93	200	10 1	14 2	83	5,640	32,000	12,200
120	331	105	206	10 7	14 5	85	5,900	34,700	14,700
130	305	114	213	11 3	14 8	87	6,130	37,000	17,000
140	284	122	218	11 9	15 0	88	6,340	39,200	19,200
150	266	127	223	12 4	15 3	89	6,520	41,300	21,300
160	250	132	227	12 9	15 6	90	6,670	43,300	23,300

A generalized yield table (Table 1) presents the essential normal-stand values. In the computations a more detailed table was used. Instead of the five broad site-quality classes shown in the table, actual site-index classes were used. Site index is an indicator of site, deduced from the average height of the dominant and co-dominant trees when the stand is 100 years of age. A set of curves similar to Figure 1 relates the height at any age to the height the stand will have at 100 years. Site-index classes of Douglas fir range from 80 to 210 feet. In Figure 1,⁶ Site I represents site index 200; Site II, site index 170; Site III, site index 140; Site IV, site index 110; Site V, site index 80. Table 1 lists three volume measurements—cubic-foot volume (the total cubic-foot volume inside bark of all the trees); board-foot volume by International rule for 1/8-inch kerf, which includes only trees of the 7-inch d. b. h. class and larger; and board-foot volume by Scribner rule, which includes only trees of the 12-inch d. b. h. class and larger.

⁶ Up to the 160-year point this graph is based upon the very large number of trees whose heights were measured in the course of the normal-yield study. In order that it might be possible to determine the site-quality class of land still covered with old-growth forests, additional measurements were made, in 40 different even-aged stands from 150 to over 400 years old, of the average height of the dominant and codominant trees, and the height-on-age curves for the five principal site-quality classes were projected.

AVERAGE DEGREES OF STOCKING

The average values of the empirical stands will be considered first and compared to the normal-yield tables. Strip samples in many second-growth Douglas fir forests gave a good idea of how much, on the average, natural forests underrun the normal. The large bare areas, or surveyable openings, were left out, and only the small holes or gaps in the Douglas fir canopy, at the most a couple of chains across, which are always found and can not be surveyed out, were included in the strip area. Table 2 lists the average relation of these actual stands to normality by five classes of data, namely, by strip samples for the first season and for both seasons, by combined tract tallies, and by two classes of selected sample plots in normal stands.

TABLE 2 *Average degrees of normality of actual stands of immature Douglas fir and of selected sample plots*

Class of data	Stand		Volume			
	All trees	12 inches d b h and over	Total basal area	Cubic measure	International rule	Scribner rule
	Number	Per cent	Per cent	Per cent	Per cent	Per cent
Strips (1926), 179 acres, average age, 82.8 years, average site index, 162.	82 4	85 5	89 4	89 2	92.3	93 7
Strips (1926 and 1927), 531 acres, average age, 77 years, average site index, 159.	77 4	77 5	80 7	80 5	82 1	83 3
Tracts (1926 and 1927), 81 tracts.	78 2	79 5	82 0	82 1	84.3	85 7
Sample plots from normal yield study, all ages, 433 plots, average 77 4 years, average site index, 155.	103 1	105 5	107 0	109 7		111 5
Sample plots from normal yield study, 70-year age class, 118 plots, average age, 75 years, average site index 150.	99 7	95 7	99 2	102.6		99 7

In the preparation of Table 2 a number of erratic values were rejected. One tract, for instance, was thrown out because it was obvious from the character of the averages that either the age or the site had been determined wrongly. Number of trees decreases, but basal area and volume increase with advance in age or improvement in site. The effect of an error in age or site determination upon the percentages of normal number of trees will therefore be exactly the opposite to the effect upon the percentages of normal basal area or volume. If, for instance, the age is overestimated, the computed percentage of normal number of trees is increased and the percentage of normal basal area is decreased. Therefore, in a study of this nature, extreme accuracy in the determination of age and site is essential. Certain strip-acres whose actual or normal board-foot volume was zero were also disregarded—temporarily, at least.

Table 2 is the source of much interesting information. It is remarkable that the average value of the tracts sampled is so high in degree of stocking. Based upon the total strip acres measured in both years, the average stand has 77 per cent of the normal number of trees, 81 per cent of the normal basal area, 80 per cent of the normal cubic-foot volume, and 82 to 83 per cent of the normal board-foot volume. These values are all appreciably lower than those

based on the first season's work alone, for the reason that in the first season the better stands were visited, and in the second season the study was extended to the poorer forests.

It must be emphasized that these averages do not apply to extensive, diversified areas, unless the meadows, barren land, other forest types, etc., are surveyed and excluded from consideration. In a survey of 4,190 acres, of which 3,700 acres were actually covered by strips, only 2,631 acres were classed as second-growth Douglas fir type. The remaining area consisted of other types, such as hardwoods (202 acres), cedar, hemlock, and white pine types (422 acres), major waterways and river flats (531 acres), and veteran stands (404 acres). Such large surveyable eliminations must be given consideration in dealing with any extensive tract.

RANGE IN DEGREE OF STOCKING

The average of Table 2 will apply in general, but individual acres and tracts may vary widely from them. To illustrate to what extent actual values can vary, there are listed in Table 3 the maximum and

TABLE 3.—Standard deviations and actual (not theoretical) maxima and minima of the values composing the averages shown in Table 2 given to indicate the range of values to be expected

Factor	Strips, 1926 and 1927				Temporary sample plots			
	Strips, 1926, stand- ard deviation	Stand- ard deviation	Total range	Range of central two- thirds of cases	All ages			70-year class, stand- ard deviation
					Stand- ard deviation	Total range	Range of central two- thirds of cases	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Total number of trees.....	26.1	24.9	14-179	52.5-102.3	29.5	40-250	73.9-132.9	26.6
Trees 12 inches d. b. h. and larger.....	21.6	26.2	51.3-103.7	41.5	64.0-147.0	25.6
Basal area.....	19.5	19.4	25-145	61.3-100.1	18.7	60-170	88.3-125.7	15.7
Cubic-foot volume.....	19.4	19.7	25-138	60.8-100.2	22.0	60-210	87.7-131.7	22.2
International board-foot volume.....	20.5	21.2	25-144	60.9-103.3
Scribner board-foot volume.....	22.8	28.0	9-286	55.3-111.3	37.6	0-550	53.9-169.1	29.5

minimum percentages of normal stocking that were found and the standard deviations from the averages. The standard deviation represents a range on each side of the average in which approximately two-thirds of the variations can be expected to be confined. For instance the standard deviation of basal area for the 1926 and 1927 strips is 19.4 per cent; its corresponding average from Table 2 is 80.7 per cent. Then in two-thirds of the instances the differences between the individual strip acre and the average lie between 80.7 plus 19.4 and 80.7 minus 19.4, or between 100.1 and 61.3 per cent. Theoretically all cases should lie between the average minus and the average plus three times the standard deviation or, for 1926-27 basal area, between 22.5 and 138.9 per cent. The actual minimum and maximum according to Table 3 are 25 and 145 per cent.

The variability for basal area, cubic-foot volume, and International board-foot volume, as shown by the percentage standard

deviation, is surprisingly uniform. However, number of trees and Scribner board-foot volume are distinctly more variable and show these factors to be subject to different influences.

There are two reasons for the increased variability of the Scribner volume. The first is that only a part of the stand is considered. The increase in number of trees 12 inches and more d. b. h. is comparatively rapid at the age when stands are getting their first 12-inch trees, and there are apt to be large percentile differences between the actual stand and the yield-table values, since a few trees can have great effect upon the total board-foot volume. Therefore, young stands or stands on poor sites can be expected to show these erratic percentages in Scribner board-foot volume. Because of this, difficulties can be expected when the values in basal area, cubic-foot volume, etc., are related to the Scribner values.

The second reason is that the Scribner rule does not accurately measure the lumber which can be produced from logs and trees and that this inaccuracy is greatest in small trees. Part of the variability which is being discussed is therefore undoubtedly due merely to errors inherent in the rule used.

Scribner rule, not being a precise unit of volume measure and being applied only to the merchantable trees, does not lend itself to analytical treatment, and almost invariably it proves itself unamenable to precise methods. Were it not for the fact that board-foot rule is a current commercial unit for expressing stand volume, it would be preferable to omit it entirely. Therefore, if undue emphasis seems to be placed upon it here, that is because much effort was spent in trying to get expressions of stand values related to this conventional unit of measure.

The failure of the percentages of number of trees to be in line with the percentages of the other factors is explained by the numerous small understory trees or persistent suppressed trees in some stands, but not in all. The effect of this variation is to render this factor useless.

These ranges in stocking percentages of strip acres are apparently large, but they are exceeded in certain cases by the ranges in stocking of square sample acres. The variation in number of trees on 433 plots is between 40 and 250 per cent, with a standard deviation of 29.5 per cent. This is very interesting in view of the fact that all the plots were chosen for their fairly normal and homogeneous appearance. Basal area shows a range from 60 to 170 per cent with a standard deviation of 18.7 per cent and cubic-foot volume a range from 60 to 210 per cent, with a standard deviation of 22 per cent. Scribner board-foot volume is entirely out of proportion, as might be expected.

If whole tract averages are taken (not listed in Table 3) instead of the strip-acre averages, the ranges are reduced somewhat, for then they vary only from 38 to 152 per cent, for number of trees, 48 to 125 per cent for basal area, 51 to 122 per cent for cubic-foot volume, and 52 to 130 per cent and 30 to 219 per cent, respectively, for international and Scribner board-foot volumes.

In this study occasional individual strip acres and possibly more frequent rectangular sample plots were actually the tallies of portions of acres put on the acre basis. As a rule plots computed upon the

acre basis give higher averages and larger deviations. In the author's opinion this does not explain away, however, the large differences which exist between strip acres and plots and does not invalidate the statement that sampling an area by strips is more reliable than by scattered plots, since it automatically covers the slight irregularities which occur continually in a stand and which are not smoothed out so effectively if square plots are taken. As a rule the error of estimate increases with the perimeter, and strips have the larger perimeter for a definite area. However, the errors of a carefully conducted survey nowhere nearly approach the errors caused by the variability of the stand. The whole is a controversial subject and can be answered finally only by actual test.

SMALL HOLES AND THEIR EFFECT UPON STOCKING

The difference between actual stocking and normal stocking is due to certain causes. First of all, the breaks in the stand, the gaps in

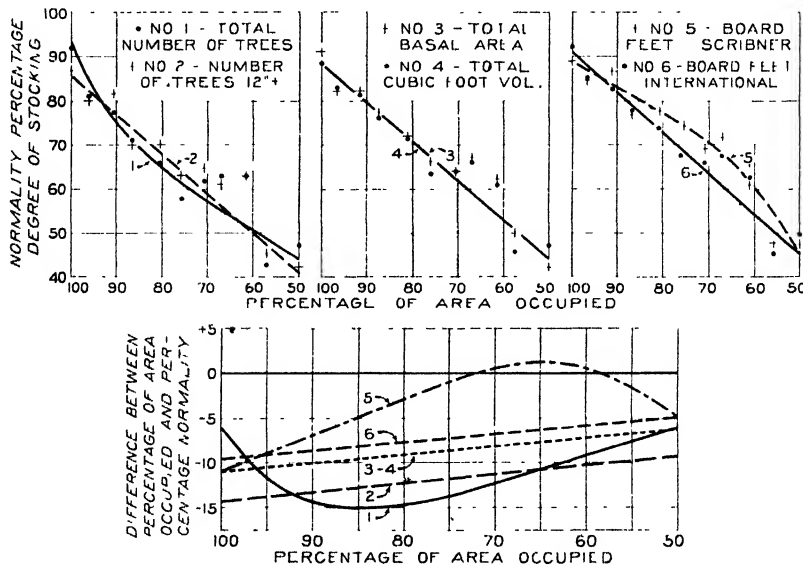


FIGURE 2—Effect of area occupied upon stocking

the canopy, or the proportion of the total area in small holes should be examined to see whether understocking consists chiefly in wide spacing of trees or in numerous small openings. The small holes or breaks in the second-growth canopy of 2 chains or less may be occupied by no trees at all, by hardwoods, by other conifers, or by veterans. The character of the stand and the size of the holes were noted on 442 strip acres; 132 of the strip acres had no portion of the area in holes, 95 had 1 to 5 per cent of their area in holes, 78 had 6 to 10 per cent, 52 had 11 to 15 per cent, and so forth, as shown in Table 4. By plotting the average stocking percentages for each group over the percentage of area occupied (lower half of fig. 2), the roughly curved values of Table 4 were obtained. When less than 70 per cent of the area is occupied, the points are apt to be erratic because of the scarcity of data.

TABLE 4.—*Relation between area occupied and stocking*

Area occupied *		Curved normality percentage					
Per cent	Acres	Number of trees	Basal area	Number of trees 12 inches and over	Volume		
					Cubic measure	International rule	Scribner rule
100	132	94	89	86	80	91	89
95	95	83	84	81	84	86	86
90	78	75	80	77	80	82	83
85	52	70	75	72	75	77	80
80	29	65	71	68	71	72	77
75	17	61	66	63	66	68	74
70	11	58	62	59	62	63	71
65	10	54	57	54	57	59	66
60	5	51	53	50	53	54	61
55	4	47	48	45	48	50	54
50	0	44	44	41	44	45	45

* 9 acres were less than 50 per cent occupied, of which 5 were less than 45 per cent.

Each one of the factors (Table 4) maintains with only minor variations a similar relation between occupied area and stocking. They all start a trifle below 100 per cent and stay within 13 per cent of each other the whole way through. In other words, the actual small openings seem to play the greatest part in the variation in understocking, while the spacing of the trees plays a less important and also less obvious rôle. Speaking generally, there seems to exist approximately a 10 per cent difference between the degree of stocking and the area occupied.

The lower part of Figure 2 shows in another way the effect of holes upon the degree of stocking. In this chart the difference between the stocking percentage and the percentage of the area occupied is plotted over the percentage of area occupied. The trends of the curves for basal area, cubic foot, International rule, and number of trees 12 inches and more are fairly parallel, rectilinear, and slowly rising. The other two curves, for total number of trees and Scribner-rule volume, are distinctly different. What plausible explanations, if any, exist for these relationships, and what light do they throw upon the character of the understocking? In the first place the gross effect of holes is eliminated and the lesser effect of tree spacing is made apparent. A low proportion of the area in small holes means a great dropping off in the total number of trees. However, since the trend in the percentage of 12-inch trees is rising, it is apparent that the trees chiefly affected are below this diameter limit, or are those of the poorer dominance classes. Increased frequency of holes, within certain limits, indicates greater canopy space for the remaining trees and hence better individual development, or, in other words, a larger proportionate number of 12-inch trees and, furthermore, a greater proportionate Scribner volume. The Scribner curve and number-of-trees curve are of similar shape but exactly inverse in position with regard to both coordinates.

CAUSES OF THE SMALL HOLES IN A FOREST

Undoubtedly the character of the stocking in relation to the area occupied depends largely upon the original cause and age of the hole. Unfortunately such relations are practically indeterminable, and all the investigator can do in a short-time study is to show the relative importance of the several agencies. For this purpose field observations were made of the probable origin of each hole. Table 5 summarizes these, dividing them into size groups and estimating the measure of their respective importance by giving an approximation of the total area covered. Out of 660 holes, 17 per cent are listed as "unknown." "Veterans" include small areas eliminated because they are occupied by veteran trees. "Fire," "insects," and "wind" holes are apparent from the present condition of the trees or stand. "Gullies and creeks" combine often with "hardwoods or wet places" where Douglas fir establishment is seldom a success and where cedar and hemlock or hardwoods take its place. "Rock outcrops and steep slopes" are sometimes contributing factors to unevenness in stocking. "Natural" holes are those apparently due to original failure in stocking, where no Douglas fir ever started.

The holes below 0.05 acre are by far the most numerous, amounting to two-thirds of the total number, although they aggregate only about one-third of the total area. By and large, 9.5 per cent of the average acre is in small holes. This estimate was obtained by an independent summing of the sizes of the holes and not from the rough classes of Table 5.

TABLE 5.—Cause and size of small holes in immature Douglas fir stands^a

Cause or nature of holes	* Small holes, classified by size of hole as indicated						Approximate total area		
	0 0-0 049 acre	0 05-0 099 acre	0 1-0 149 acre	0 15-0 199 acre	0 2 acre and over	All holes			
	Number	Number	Number	Number	Number	Number Per cent	Acres	Per cent	
Fire.....	35	8	2	1	46	7	1.91	6	
Veterans.....	45	13	5		63	10	2.72	9	
Insects.....	34	9	1		44	7	1.65	5	
Wind.....	62	14	4		80	12	3.10	10	
Gullies and creeks	51	30	7	2	90	28	4.76	37	
Hardwoods or wet places.....	44	33	12	7	97		6.58		
Rock outcrops and steep slopes.....	25	4	2	1	32	5	.66	2	
Natural.....	43	14	6	4	67	10	3.58	12	
Unknown.....	87	21	4	1	113	17	4.44	15	
Miscellaneous.....	19	9			28	4	1.16	4	
Total	445	155	43	16	1	660	100	30.58	100

^a Of the 63 tracts from which these data are taken, 29 show traces of fire, 28 show no traces, and 6 are doubtful.

Gullies and creeks plus hardwoods form by far the most important combined factor both in number of holes and in area. Together they constitute 28 per cent of the holes and 37 per cent of the total area in holes. They even slightly outrank in number the sum of natural and unknown holes. Wind is the next most important factor with 12 per cent in number of cases and 10 per cent in area. Presence of veterans in the stands (10 per cent of the holes) ranks next, with damage by fire (7 per cent) and insects (7 per cent) following.

Other minor causes include rock outcrops and steep slopes (5 per cent), and miscellaneous (4 per cent), which include small cuttings and roads.

The fact that fire ranks so low in causing small holes is noteworthy, especially since 29 tracts out of 63 described show clear traces of fire. Despite its pronounced thinning agency in much younger stands than those studied and its importance as a destroyer of whole forest stands, fire is not a prominent cause of small holes. Clean burns caused by crown fires, not the light surface burns which thin out young stands, are a prevalent cause of extensive gaps in a forest. Failure of man-made openings to restock properly is another. Both are of overwhelming significance but do not find much place in the present discussion on understocking of stands. The character and quantity of such openings are different for each locality and average values would have no utility. In this study the strip-survey lines were stopped when such openings were encountered.

EFFECT OF UNDERSTOCKING OR OVERSTOCKING ON METHOD OF YIELD PREDICTION

An important part of this study is to determine the actual effect of understocking or overstocking upon such stand values as number of trees, basal area, or volume. The analysis will give an idea of the best index of stocking and its relation to other values. If basal area is found to be a reliable index of stocking, will cubic-foot volume or board-foot volume have exactly the same normality percentages as basal area? If not, what significance can be attached to the difference, and what effect will this difference have on yield predictions? The chosen factor should be relatively simple so that reliable indications of volume normality will be obtained with the minimum of computation.

Stocking, or the degree of normal condition, can be expressed in terms of basal area, number of trees, or any of the volume values. Ordinarily one considers a heavily stocked forest one in which the trees are so close together as to form a complete, dense canopy. The other definitions of stocking deal with secondarily derived factors—for instance, with basal area, for which a diameter tally is needed, and with volume, for which a height curve in addition to the diameter tally is necessary. Undoubtedly an index based upon volume itself is most accurate. However, with volume taken in its broad sense, the relation would vary greatly, depending chiefly upon the product for which the stand is being raised—whether for pulp wood, for ties, for poles, for boards, or for timbers. Many of these expressions of stocking in terms of merchantable products are of transitory value only and can easily be omitted from consideration.

The method of testing whether number of trees or basal area is the better index of stocking may be based first upon a curve of stocking in percentages of volume, averaged in 10 per cent classes, over number of trees or basal area. A smooth logical trend will be an evidence of relationship. Statistical measures will be computed, such as the correlation coefficients and the standard errors, the regression lines will be plotted, and certain correlation indices and modified standard errors will be found. In this way the relationships which give the best-fitting regression lines or modified curves, as evidenced

by high correlation coefficients or indices and low standard errors, will be those from which the most suitable index of stocking is obtained.

CORRELATIONS

Table 6, presenting standard deviations and correlation and alienation coefficients for the surveyed strips, facilitates further reference and avoids undue repetition. The correlation coefficient is an indication of straight-line relationship between two variables. Complete association is signified by 1, therefore the closer a coefficient is to 1 the better is the relation. The alienation coefficient, on the other hand, measures the actual proportion of variation attributable to other causes, including curvature of the relation between the two in question.

TABLE 6.—Standard deviations, correlation coefficients, and alienation coefficients computed for surveyed strips in actual stands

Factor	Average stock- ing	Stand- ard devia- tion	Rela- tion ^a	Number of trees 12 inches and over	Basal area	Cubic measure	Volume	International rule	Scribner rule
	<i>P. et</i>	<i>P. et</i>							
Number of trees....	77.4	21.9	C. C.	0.377±.025 (.926)	0.613±.018 (.790)	577±.020 (.817)	473±.023 (.881)	120±.029 (.993)	
Number of trees 12 inches and over in diameter	77.5	26.2	C. C.	—	.656±.017 (.755)	662±.016 (.750)	749±.013 (.663)	847±.008 (.532)	
Basal area.....	80.7	19.1	C. C.	—	—	955±.003 (.207)	926±.004 (.378)	685±.016 (.720)	
Volume, cubic feet	80.5	19.7	C. C.	—	—	—	.935±.004 (.753)	.658±.017 (.532)	
Volume, International rule	82.1	21.2	C. C.	—	—	—	—	832±.009 (.555)	
Volume, Scribner rule.	83.3	28.0	C. C.	—	—	—	—	—	

^a The correlation coefficient (C. C.) is given plus or minus the probable error of the coefficient, which is $0.6745 \frac{1-r^2}{\sqrt{n}}$ (n being 531). The alienation coefficient (in parenthesis) equals $\sqrt{1-r^2}$, where r is the correlation coefficient. Alienation coefficient (A. C.) = $\frac{SE}{SD}$ = SD about curve.
SD = SD about mean.

NUMBER OF TREES AS AN INDEX OF STOCKING

First let the criterion of number of trees be examined, since it would be the simplest index if workable. All the values have been previously converted to percentages of the normal yield table values. In Figure 3 the average points of each 10 per cent group are plotted over the percentages of number of trees as abscissas, and the regression lines (mathematically computed lines of average trend) are drawn through them. If the several plottings are superposed they show very similar trends. The points indicate a sharp rise from 0, passing between 60 and 80 per cent values at 40 per cent normal number of trees, then increasing gradually to a maximum of 100 to 110 per cent at about 120 per cent normal number of trees, after which there is an apparent dropping off. The data beyond 120 per cent, however, are too few for accurate conclusions.

Trends of this nature indicate that, in general, volume per cent at first rises rapidly with an increasing relative number of trees,

soon slows up, reaches a maximum, and then falls off with further increase in number of trees. In other words, there seems to be an ideal number of trees for maximum volume. This number, moreover, being at least 120 per cent, strongly suggests that the conception of normality involved in the yield tables is somewhat conservative. However, graphical representation alone is insufficient and statistical measures must be employed to show whether the relations are trustworthy.

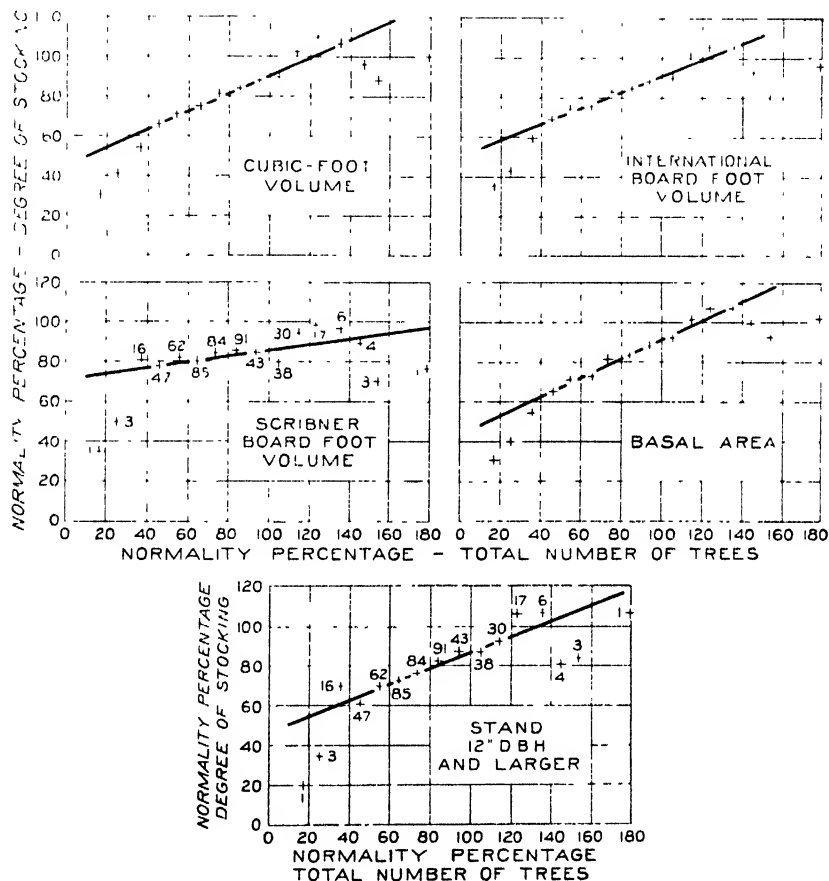


FIGURE 3.—Degree of stocking with respect to volume and number of trees 12 inches and larger, related to total number of trees as an index of stocking. Straight lines represent computed regression lines

The correlation coefficients (Table 6) indicate very well how weak the relations are. The coefficients between degree or normality of number of trees and number of trees 12 inches in diameter and larger, basal area, cubic-foot volume, international board-foot volume, and Scribner board-foot volume are 0.377, 0.613, 0.577, 0.473, and 0.120, respectively. The plus or minus quantities after the coefficients of Table 6, indicating the probable errors of the coefficients, are in every case very small and negligible. The respective

alienation coefficients are 0.926, 0.790, 0.817, 0.881, and 0.993, all of which are percentages of variation due to other factors and to curvature if present, as explained previously. Because of the small number of weights in the portions of the average curves which deviate in a pronounced manner from the regression line, for instance from 0 to 30 per cent and from 130 to 180 per cent, the correlation indices of the fitted curves would probably be improved by less than 0.10 increase. Correlation index is to a fitted curve what the correlation coefficient is to the regression line. The number-of-trees relation with Scribner volume is amazingly weak, altogether too weak for further use, although one might reason that decrease in number of trees would be accompanied by increased size of the individual trees, hence increased board-foot volume.

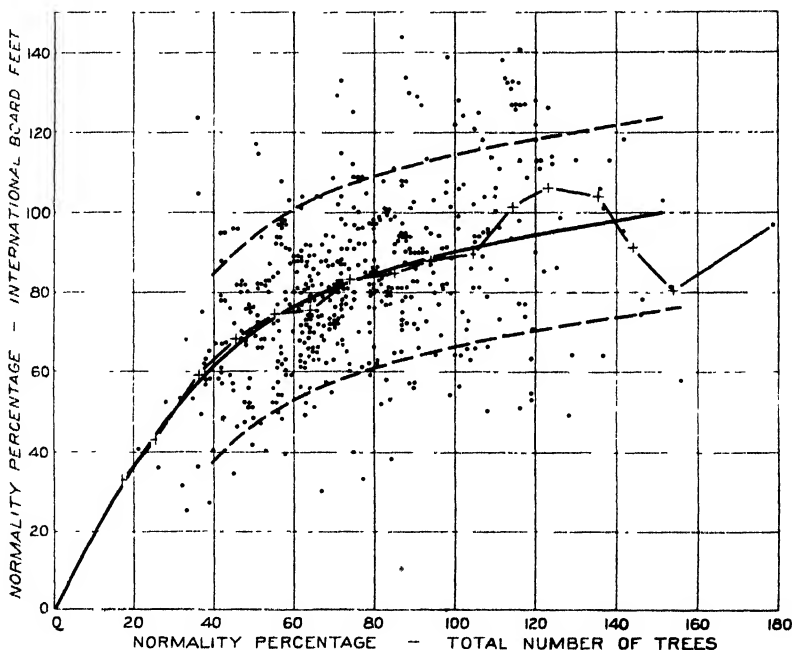


FIGURE 4.—Dispersion of normality percentages of International board-foot volume with normality percentage of total number of trees as a basis for stocking

By taking the coefficient between total number of trees and volume by International rule (0.473) and reproducing the actual points in addition to the average values of Figure 3, an array such as that presented in Figure 4 is obtained. This bears out graphically the contention that the correlation coefficient could not be improved very much by introducing curvature to the straight-line regression. The band of points is very wide, and there would be little benefit in any further attempts to use the percentage of normal number of trees as an index of stocking, especially since from Table 6 it is very apparent that other relations exist which have much greater value. In a preliminary test of a portion of the data (1926 data), improvement of the number-of-trees relation to Scribner volume was sought by

fitting a curve, but the correlation index showed an increase of only 0.03 over the correlation coefficient for the straight line regression.

An additional method of showing the weakness of the number-of-trees percentage in relation to International-volume percentages of Figure 4 is to compute the standard error around the curve, plot it, and notice how wide a belt it forms. This is done assuming that the correlation coefficient is not much improved by drawing the curve. The alienation coefficient for the correlation coefficient 0.473 is 0.88. Since the alienation coefficient is equal to the standard error over the standard deviation ($AC = \frac{SE}{SD}$) and the standard deviation for international volume is 24.9 per cent, the standard error is 21.9 per cent. Actually standard error is a little smaller than this, depending upon the improvement made by passing the curve through the points instead of using the regression line. The broken-line curves on either side of the average curve represent the range in which two-thirds of the cases can be expected to lie.

Why should total number of trees show such a poor connection with stocking? The explanations undoubtedly are that it is hard to eliminate the trees which do not really belong to the main stand, and do not comprise most of the basal area and volume, that the trees are unevenly spaced, and that many relatively small trees persist or do not persist. These irregularities affect the percentages of number of trees but do not appreciably affect the percentages of basal area or volume.

BASAL AREA AS AN INDEX OF STOCKING

Basal area is the next factor to be considered, since the simple factor of total number of trees fails to be significant. The stocking percentages for board-foot volume by Scribner rule and by International rule and for total cubic-foot volume are plotted upon the stocking percentages for basal area. (Fig. 5.) The plottings of all three follow the same trend, remaining within 5 per cent of each other practically throughout. The 45° angle which they make with the axes affords a very favorable trend for prediction purposes. Indicated curvilinearity is very slight. Consequently the improvements to the correlation coefficients of Table 6 would be small if curves were fitted. The correlation coefficients for International board-foot volume and for cubic-foot volume with respect to basal area are high, but even a small improvement would make the relations more perfect. Board-foot volume by Scribner rule again seems to be more erratic, with the result that basal area is not as good an index of Scribner stocking as desired. This weakness will undoubtedly be a source of error in making yield predictions for board-foot volume by Scribner rule by means of stocking percentage founded on basal area. The cubic-foot volume predictions and the International board-foot volume predictions will, however, be much more reliable. The coarse-scale graphs (fig. 4) do not permit determining the relationships with sufficient accuracy. A table of values read from the original large-scale figures giving the best curved values, not necessarily the regression values of Figure 5, is drawn for further use. (Table 7.)

TABLE 7.—Relation of the normality percentages for board-foot volume and cubic-foot volume with basal area as index of stocking

Basal area	Cubic-foot volume normality	Board-foot volume normality by—		Basal area	Cubic-foot volume normality	Board-foot volume normality by—	
		International rule	Scribner rule			International rule	Scribner rule
Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
10	10	10	8	110	109	110	113
20	20	20	19	120	118	119	122
30	30	30	30	130	127	128	131
40	40	40	41	140	136	136	140
50	50	51	52	Correlation coefficient	0.955	0.926	0.685
60	60	61	62				
70	70	71	73	Alienation coefficient	.297	.378	.729
80	80	81	83				
90	90	91	92	Standard error	5.9	8.0	20.4
100	100	101	103				

The alienation coefficients for basal area with respect to volume in cubic feet, International board feet, and Scribner board feet are 0.297, 0.378, and 0.729, respectively. The corresponding standard

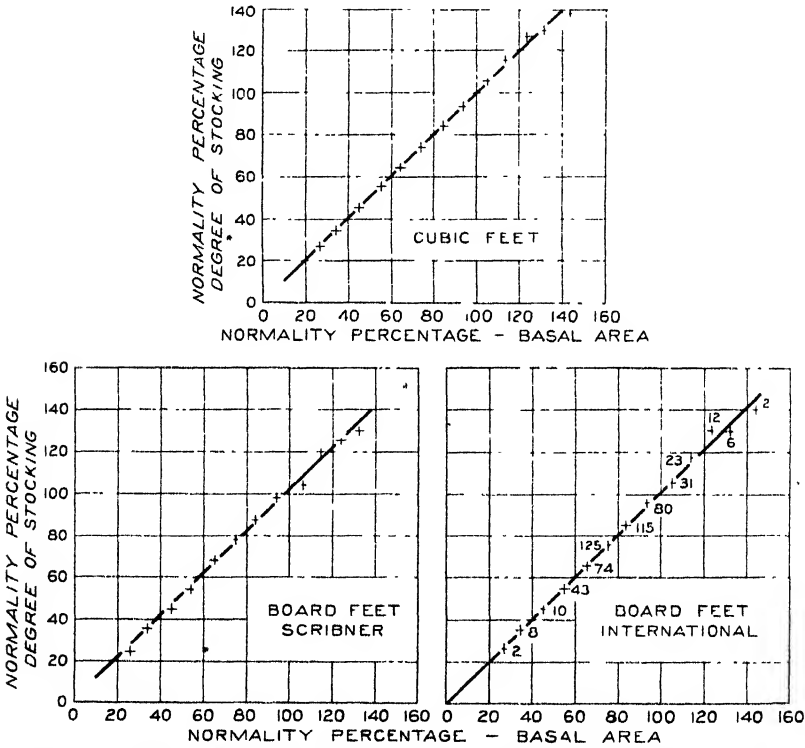


FIGURE 5.—Degrees of stocking with respect to cubic-foot volume and board-foot volume related to basal area as an index to stocking. Straight lines represent computed regression lines

errors are stated in Table 7 as 5.9, 8, 20.4 per cent. The 8 per cent error makes a very favorable contrast to the 21.9 per cent that was

obtained when total number of trees was used as an index for identical board-foot volume. The Scribner relation, although it forms a nice line of averages (fig. 5), has not been so favorable a standard error.

Basal area, therefore, with its good correlation coefficients in respect to cubic-foot volume and International board-foot volume, its resulting low standard errors, and its convenient form of relationship (a 45° angle), is satisfactory. If a better index for Scribner board-foot volume is still desired, other factors must be examined.

NUMBER OF TREES 12 INCHES AND OVER AS INDEX OF BOARD-FOOT VOLUME

Throughout the computations those dealing with board-foot volumes by Scribner rule are always the most variable, contain the most erratic values, and are the most unsatisfactory to analyze for consistent, valuable relations. So far it has been shown how poorly both total number of trees and basal area are correlated with Scribner-rule volume. In seeking another stronger relationship it must be remembered that estimates of board-foot volume of small timber by the Scribner rule are misleading unless accompanied by an estimate of the overrun percentage. If this could be determined and applied, a volume in board feet would result which would differ little from the volume by the International rule, but, as a matter of fact, this overrun percentage is itself very variable. For those wishing the Scribner volume, the best indicator proves to be the percentage of normal number of trees 11.6 inches and more d. b. h., since, according to Table 6, the correlation coefficient between the two is 0.847.

Figure 6 shows graphically that a certain degree of curvilinearity exists. The erratic points beyond 140 per cent, being of little use, are eliminated. They represent chiefly values read from the relatively weak portions of the yield tables—values for the low ages or the poor sites, where number of 12-inch trees is small and volume by Scribner rule is slight, and where any small exceptional condition would disturb the percentages greatly. Setting the maximum stocking percentage for number of 12-inch trees at 140 per cent will reduce the correlation coefficient for Scribner volume from 0.847 to 0.800, but will increase that for cubic-foot volume from 0.662 to 0.728 and that for International volume from 0.749 to 0.787. It is evident that a curve will express the relation between number of 12-inch trees and volume by Scribner rule much more accurately than the regression lines, especially in the low and high percentages where the plotted points deviate pronouncedly from the regression line. Table 8 gives these readings, together with a separate set of readings from a curve drawn in exactly similar fashion except that the individual acres are first grouped and are averaged by the tracts in which they were taken.

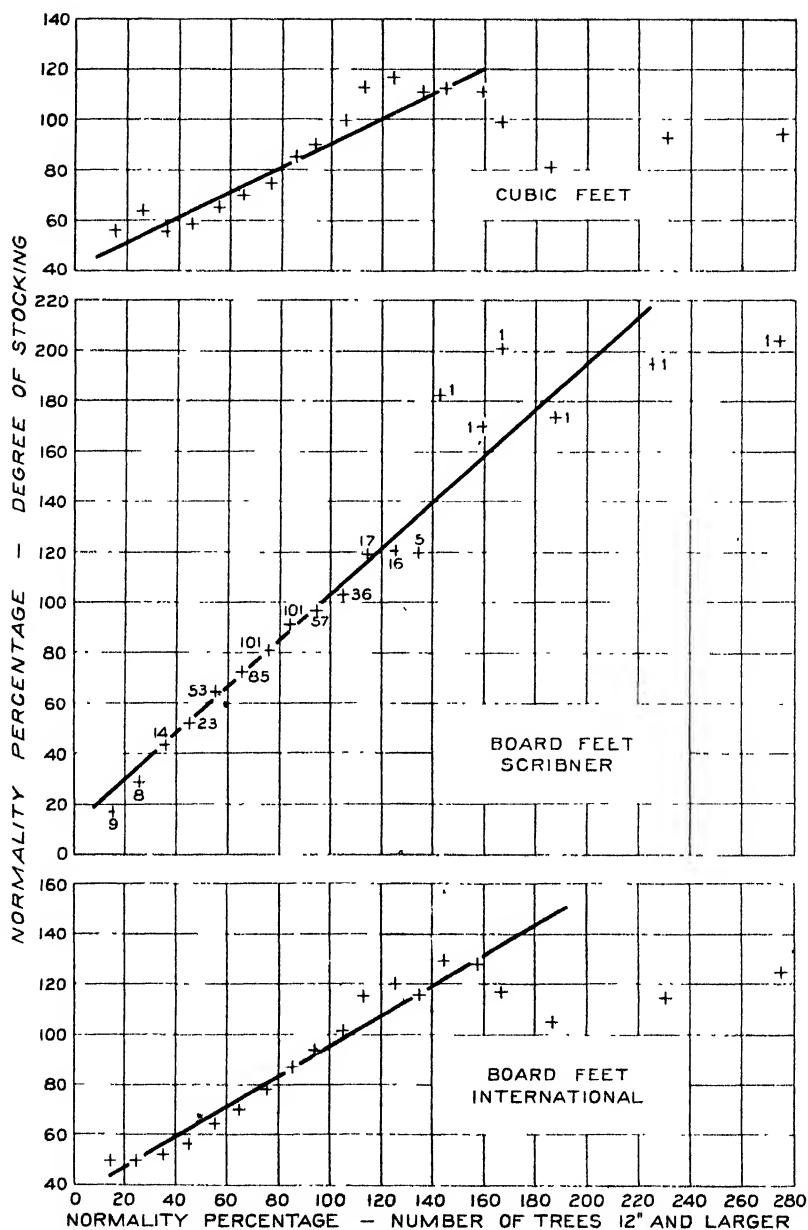


FIGURE 6.—Degrees of stocking with respect to volume related to number of trees 12 inches and larger as an index of stocking. Straight lines represent computed regression lines

TABLE 8.—*Relation of the normality percentages for board-foot volume by Scribner rule with number of trees in the 12-inch d. b. h. class and larger as index of stocking*

Volume normality in board feet, Scribner rule on			Volume normality in board feet, Scribner rule on—		
Number of trees 12 inches and larger			Number of trees 12 inches and larger		
	Individual strip acres	Tracts		Individual strip acres	Tracts
<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
10	10		100	103	101
20	22		110	111	108
30	31	30	120	118	115
40	46	44	130	125	121
50	58	56	140	132	128
60	68	67	Correlation coefficient	0.800	0.771
70	78	77	Correlation index	837	.838
80	87	85	Alienation index	547	546
90	95	93	Standard error	13.7	11.8

For the main portion of the curves between 50 and 100 per cent the differences between the two sets are not at all striking. The inconsistency in the extremes is easily explained by the small number of tracts having average values within these ranges. This relation for board-foot volume by Scribner rule is easily applied and practical, especially since many of the cruises in second-growth Douglas fir are being made to a minimum diameter limit of 12 inches. The fact should be noted that the volume percentages overrun percentages of the number of 12-inch trees in the main range by 3 to 8 per cent. Due corrections must be made for this overrun in computing the yield predictions where this index of stocking is used.

The number of trees in the 12-inch class obviously can not be used as an index of stocking in young stands. It should be used only after there is a fair proportion of trees of this size, probably 50 per cent or more, and then with much caution and with the full understanding that results are not absolutely accurate and that there still exists a large chance for error in individual cases. With any other species except one which attains large sizes in relatively short time, it will be of no use at all. The best that can be said of this relation is that it is the closest found in all those tested. Why should an index involving number of trees 12 inches and larger be usable when total number of trees gives no reliable indication? It is because the variable number of small trees of the understory and in thickets of suppressed growth below 12 inches d. b. h. are eliminated by a 12-inch diameter limit and only trees with a merchantable volume are included.

RELIABILITY OF THE INDICES OF STOCKING

The dependability of these two chosen indices of stocking, basal area, and number of 12-inch trees is indicated in another way by stating the amount of error which is liable to occur in using the relations for single acres. This error, the standard error, has been mentioned before. The method of determining it corresponds to the method of computing the standard deviation except that the

regression line or fitted curve is used as a base instead of the mean of the data. It is most easily computed from the correlation coefficient and standard deviation by means of this formula—

Standard error=standard deviation \times the square root of
(1-correlation coefficient squared), or—

$$SE=SD\sqrt{1-C^2}.$$

Tables exist for the quantity under the radical sign.⁷

A range plus and minus of one standard error from the curve value includes about two-thirds of all the possible cases that are likely to be found; a range plus and minus of twice the standard error includes 95 per cent of all the cases. In Table 8 it is seen that the standard error for the relation of stocking in percentage of number of trees 12 inches and larger to volume by Scribner rule is 13.7 per cent. If number of trees is used as the base, the standard error for Scribner volume percentages will be 27.8, and if basal area is used it will be 20.4 per cent (see Table 7), showing that of these three relations the first is by far the most valuable. The relation between basal area and cubic-foot volume has a standard error of 5.9 per cent, and that between basal area and volume by international rule has one of 8 per cent; both of these are comparatively small and indicate a close relationship. With total number of trees as the basis, similar standard errors for cubic-foot volume and International board-foot volume are two to three times as great (16.1 and 18.7 per cent).

An instance of the significance of these standard errors may be cited. If on a single acre the total basal area is 60 per cent of the normal for that particular site and age, then the percentage of cubic-foot volume in two-thirds of all expected cases will lie between approximately 54 per cent and 66 per cent of the normal yield table value (60 ± 5.9), or, in other words, for a single instance there is a two out of three chance that it will be within 5.9 per cent of 60 per cent. In case the total number of trees 12 inches and larger is 60 per cent of the normal, then in two-thirds of all cases the normality percentage of board-foot volume by Scribner rule will lie roughly between 54 per cent and 82 per cent; according to Table 8, 60 per cent in number of 12-inch trees corresponds to 68 ± 13.7 per cent of Scribner volume.

These ranges of values apply to single acres only; the more acres taken the smaller will be the expected total error. For instance, if on 1 acre the standard error is 5.9 per cent, then on 100 acres of tally

the standard deviation of the average will be only $\sqrt{\frac{.059}{100}}$ or approxi-

mately 0.6 per cent. Thus, if the 60 per cent normal basal area is an average of 100 strip acres, then there are two chances in three that this average is within 0.6 per cent of the true average cubic-foot normality of the 100 acres.

⁷ MINER, J. R. TABLES OF $\sqrt{1-(r)^2}$ AND $1-(r)^2$ FOR USE IN PARTIAL CORRELATION AND IN TRIGONOMETRY. 49, p. Baltimore, Md. 1922.

RELATION OF OTHER FACTORS TO BOARD-FOOT VOLUME BY SCRIBNER RULE

After the original data had been compiled and analyzed, and after it was seen how good an index to total cubic-foot volume and International board-foot volume basal area was, and how relatively good an index to Scribner board-foot volume the number of trees in the 12-inch d. b. h. class and larger was, it was suggested that the basal area of such trees might be a still better index. The suggestion is undoubtedly valuable and this index should be tested at some future time.

It is extremely unfortunate that no more certain index of volume in board feet by Scribner rule was discovered. One suggestion is that the use of two factors instead of one would help. Work was done along this line with the 433 sample acres taken in the course of McArdle's yield study. The strongest multiple correlation coefficient of three factors, of which Scribner volume was one, involved total number of trees, number of trees 12 inches and larger, and, of course, board-foot volume by Scribner rule. The coefficient of correlation amounted to 0.899. Methods of curvilinear multiple correlation were applied as far as the third approximation, and the improvement in the correlation coefficient was then tested. The new correlation index amounted to 0.904, hardly an appreciable improvement. Further manipulation evidently would not produce more certain results, and the attempt was dropped at this stage. Even omission of the erratic values did not help much. The coefficient of 0.904 expresses, of course, a close relation; but, since the original standard deviation for this material was extraordinarily high (57.6 per cent, see Table 3), the final standard error was still too large for practical use.

Another test was made to determine what factors really influence Scribner volume. The underlying idea is based upon partial correlation in which the relation between two factors is first studied without considering the effect others may have; then other factors which may have some effect are introduced one at a time, and the change in the correlation coefficient is noted. A pronounced change indicates that the introduced factor plays some part in the relation. When the relation between the stocking percentages of number of 12-inch trees, board-foot volume by Scribner rule, total number of trees, and total basal area are taken in this way, the results of the computation are as listed in Table 9.

TABLE 9.—*Partial coefficients of correlation showing the effect of basal area, total number of trees, and number of trees 12 inches and larger, upon board-foot volume by Scribner rule*

Correlation 0 order	Correlation first order	Correlation second order
Scribner, number of trees 0.120	With 12-inch trees constant -0.4048	With both constant... -0.7109
Scribner, basal area 0.685	With basal area constant..... -0.5210	With both constant... 0.6583
Scribner, number of trees 12 inches and over 0.847	With number of trees constant..... 0.7794	With both constant... 0.8224
	With 12-inch trees constant..... 0.3225	
	With number of trees constant..... 0.8720	
	With basal area constant..... 0.7230	

Four factors, composing three pairs, are considered. In each pair one of the other factors is introduced by assuming it to be held constant while the original two are allowed to vary. Thus the original relation between Scribner volume and total number of trees is expressed by the coefficient of 0.120. If the number of 12-inch trees be held constant, then the coefficient changes to -0.4048 ; in other words, if the number of 12-inch trees remains the same then the percentages of Scribner volume varies inversely with the total number of trees; if the basal area is held constant, a similar effect is obtained. By holding both constant the reasonably large coefficient of -0.7109 is obtained. Another pair of factors, basal area and volume by Scribner rule, shows an increase if the total number of trees be held constant (a decrease if the number of 12-inch trees be held constant). That the two neutralize each other is seen from the final coefficient. The relation between the third pair, Scribner volume and number of 12-inch trees, is found to be fairly independent of the other two factors, as is evident from the fact that the coefficient is only slightly affected by holding one or the other factor constant. This in itself argues for the adoption of such a criterion for judging the normality of board-foot volume by Scribner rule, because it furnishes an independent relation.

It is recommended, in making short-term yield predictions, that basal area be used as index of stocking for cubic-foot volume and International board-foot volume, and that the normality of number of trees 12 inches and larger be used as index for Scribner board-foot volume. In long-term predictions, however, basal area should be used as the index for all classes of volume.

ADVANCE FROM UNDERSTOCKING TO A MORE NORMAL CONDITION

Up to this point no reference has been made to the possibility that the degree of normality might change with the passage of time, especially if stocking is based upon total number of trees. Many persons have believed that the more open the stand—that is, the fewer the trees—the smaller would be the loss in numbers through mortality and the better would be the individual growth of the trees; stands would become nearly normal in character if sufficient time elapsed. Also, it was urged, the fewer the trees and the better the individual development the relatively larger would be the percentages of basal area and volume with respect to the percentage of total number of trees. This condition in itself could be taken as evidence that the stands were approaching normality. Based upon such an argument, Figure 3 would indicate that approach to normality was a very real condition if the dispersion of the plots around the average curve were not considered. But, as explained before, each curve really represents a wide belt from which, if appropriate choice be made, any theory can be proved. It has been shown that an index to degree of normality must be referenced to basal area or number of 12-inch trees rather than total number of trees.

Very little can be said about the changes with passage of time in the normality percentage of number of trees 12 inches and larger. If a stand has an unduly large number of 12-inch trees, it may maintain this advantage for at least a number of years.

More direct observations upon the change which is likely to take place in an understocked forest have been obtained in the study of growth on permanent sample plots,^{*} some of which have been under observation for 15 years. Because of the small number of plots (12), the percentages are somewhat erratic. When estimated in terms of basal area the rise in degree of stocking of understocked plots in a 5-year period is 2.7 per cent; with cubic-foot volume, 4.5 per cent; with volume by Scribner rule, 2 per cent. This is so small and uncertain a correction, and is so overshadowed by larger and more important variables, that it may often be most practicable to ignore it.

Since this percentage is small, always erratic, always affected by the least irregularity in mortality rate, and unreliable in its computation even on permanent sample plots, it is certain that not much progress can be made in determining advance toward normality by studies of plots or strips measured but once. In such studies past or future diameters and height curves must be computed or drawn, allowances must be made for mortality, or whole diameter groups must be dropped out entirely to compensate for mortality.

There are a sufficient number of permanent plots in coniferous forests which have been measured for several periods and from which, by relatively little study, a good idea can be obtained of the approach to normal condition with the passage of time. In Douglas fir at least 32 plots are being measured periodically and more are being added each year.

ANALYSIS OF TEMPORARY SAMPLE PLOTS

Chiefly for academic rather than practical interest, this study was extended beyond the method of analyzing strip samples to that of analyzing plot samples. A set of 433 plots was chosen from the normal yield-study data. These plots were selected to represent equally all age classes and all site classes. In order to eliminate the effect of age, a set of 118 plots in the 70-year age class was used.

EFFECT OF SLOPE UPON DEGREE OF STOCKING

A strip system of sampling can not give information on the effect of slope because there is too much variation. On single plots, however, a certain degree of slope can be chosen and the effect of slope on stocking can be effectively defined. Figure 7 gives graphically the results of this portion of the study, and Table 10 lists the readings corrected for the average—that is to say, since the average basal area, cubic-foot volume, and Scribner board-foot volume for the 433 plots amounted to 107 per cent, 109.7 per cent, and 111.5 per cent, respectively, of the normal values, the readings of the curves had to be reduced by an amount equal to 7, 9.7, and 11.5 per cent, respectively. These degrees of overstocking of a random choice of 435 plots probably resulted from the elimination of irregular plots, which would tend to raise the averages.

^{*} MEYER, W. H. RATES OF GROWTH OF IMMATURE DOUGLAS FIR AS SHOWN BY PERMANENT SAMPLE PLOTS. *Jour. Agr. Research* 30: 193-215, illus. 1928.

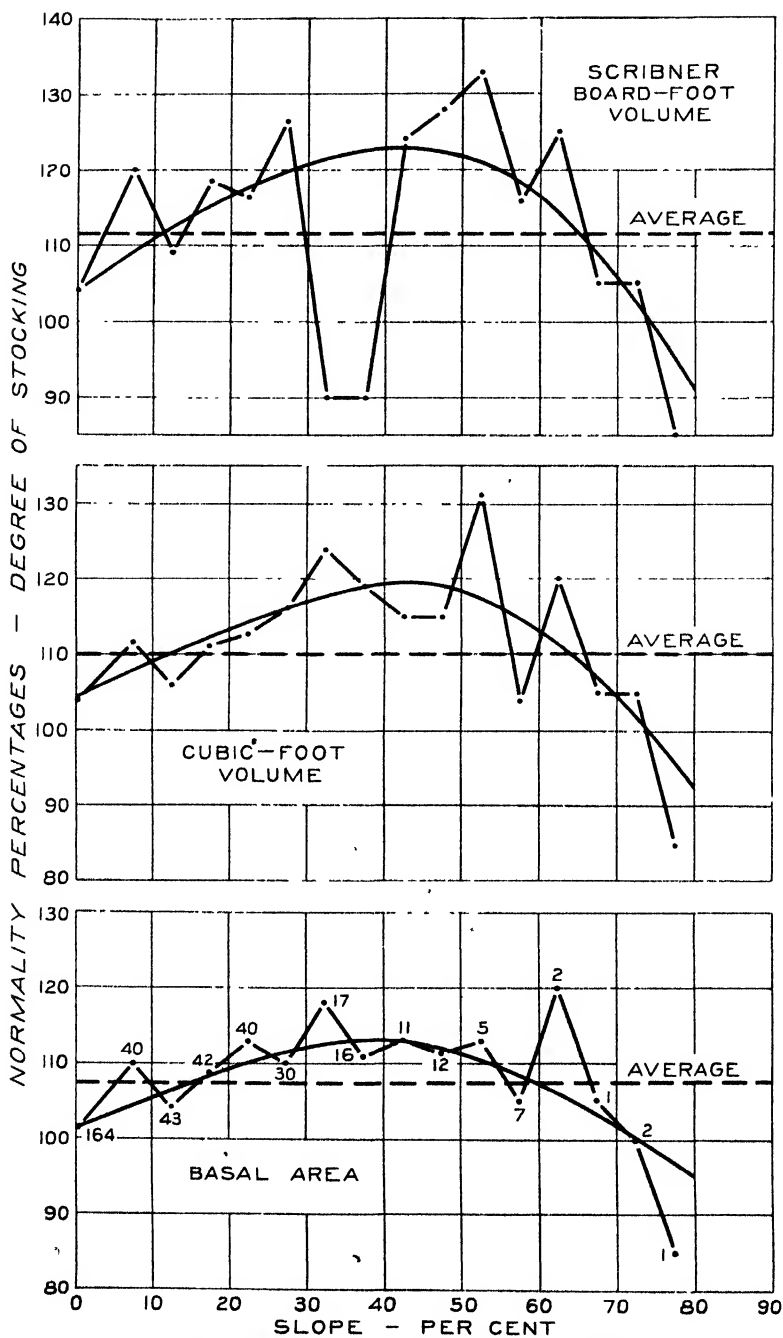


FIGURE 7.—Effect of slope upon degree of stocking

TABLE 10. - *Effect of slope upon the degree of stocking*

Slope	Correction				Basis, plots	Slope	Correction				Basis, plots
	Basal area	Volume					Basal area	Volume			
		Cubic feet	Board feet (Scribner)					Cubic feet	Board feet (Scribner)		
<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Number</i>		
0	91	95	92	164	50	104	108	111	17		
10	98.5	99.5	99	83	60	100	103	105	9		
20	102	103.5	104.5	82	70	95	91.5	94	3		
30	105	107	109	17	80	88	82.5	80	3		
40	106	109.5	111.5	27							

According to Table 10 the maximum yields are produced on slopes of 40 per cent. Even if the acres were transposed to surface measure instead of using horizontal measure, as is done throughout this study, slopes of about 30 per cent would still be indicated as producing a maximum yield. The percentages have been found to be conservative; values for the 70-year class, for example, are still larger. From the shape of the curves, low correlation coefficients can be expected. A much improved correlation index should be obtained if a curve is fitted to the plotted points. This test was applied to the 118 plots of the 70-year-age class. The correlation coefficient between slope percentage and basal-area normality was only 0.186, but the correlation index of the fitted curve was 0.922, a great improvement. The correlation coefficient between slope percentage and Scribner-volume normality was 0.307; the correlation index was 0.160. Much closer relationships than are indicated by these coefficients probably exist, but they are obscured by the wide range of stocking in the original material.

The explanation of this effect of slope upon the yield probably lies in the increased amount of light the individual tree receives as a result of the "staggering" of the tops. The significance of the effect of the slope on stocking is somewhat veiled by the varied topography on many areas.

EFFECT OF ASPECT UPON DEGREE OF STOCKING

Intimately connected with the question of slope is that of aspect. McArdle,⁹ in his study of normal yields, concludes that the best yields are obtained on north and east slopes. No very definite conclusions could be reached in the course of strip surveys, since a strip can cover a number of changes in aspect. However, general observations gave definite support to McArdle's statement. On the steeper south and west slopes, at least, the site qualities are continually changing with each break in topography, each draw, each scabby ridge. The past events in the history of the area surely have had some effect; these hotter slopes may have been burned more fiercely and have had a greater impoverishment of their soil than the moister north and east slopes. In critical situations a successful restocking can not be expected, since the surface soils dry out too quickly after the cessation of the winter and spring rains.

⁹ See footnote 2.

EFFECT OF SITE UPON DEGREE OF STOCKING

The effect of site upon the stocking of a stand has proved quite indeterminate, although all groups of data were subjected to analysis. If the poorer sites have lower degrees of stocking, the data do not show it. The reader must keep in mind, however, that the discussion is still of normality percentages and not of absolute values.

COMPARISON OF PLOT METHOD WITH STRIP METHOD

A very interesting comparison is that of the relations of stocking obtained by strip analysis with those obtained by plot analysis. The preparation of tables similar to Tables 6, 7, and 8, and figures similar to Figures 3, 4, 5, and 6, although it led to no new conclusions, brought out at least one astonishing and disturbing condition—the increased variability of some of the relations and the poorer correlations. One might expect that picked plots would exhibit less variability, since they are chosen as well-stocked areas; but actually a strip automatically irons out many inconsistencies that are maintained in a square plot of the same acreage. The size of the plots also has something to do with their variability, since small plots put on the acre basis will show a greater range of values and sometimes higher averages than full-acre plots. In this study, however, no plots are below 0.25 acre in size and a great many are 0.5 to 1 acre. The standard deviation of basal area remains about the same, being (according to Table 3) 19.4 for the strips, 18.7 for the 43 plots, and 15.7 for the 118 plots of the 70-year-age class. The widest difference occurs in the normality percentages of board-foot volume by Scribner rule. The 433 plots have a standard deviation of 57.6 per cent as compared to 28 for the strips and 29.5 for age class 70. The young forests and the poor sites, where Scribner volumes are small and chance for percentage errors large, are partially the cause of the difference.

In their essential points the relations between stand factors are surprisingly alike, whether computed by strips or by plots. The more material taken, the more uniform will the results be. Based upon the strip surveys taken during the first season alone, certain differences were noticed and significance attached thereto, but after the second season's investigations were added the differences were ironed out and lost all significance. For example, with regard to the dependence of normality of board-foot volume by Scribner rule upon that of number of trees 12 inches and larger, the final analysis of the strips gave a regression equation of the following form—

Scribner volume per cent = 0.905 (percentage of trees 12 inches and over) + 13.2 .
To this is compared the regression obtained by taking 433 sample plots—

Scribner volume per cent = 0.930 (percentage of trees 12 inches and over) + 9.9
or that of the 118 plots of the 70-year-age class—

Scribner volume per cent = 0.864 (percentage of trees 12 inches and over) + 17.1 .

This agreement is remarkable considering that three distinct groups

of material are treated. When curved over the actual points the agreement is still more perfect.

The relation between normality of total cubic-foot volume and that of total basal area is not quite so good, but still the differences are not pronounced enough to overthrow any conclusions.

For strip surveys: Cubic-foot volume per cent = 0.972 (basal area per cent) + 2.1.

For 433 plots, all ages: Cubic-foot volume per cent = 0.917 (basal area per cent) + 10.4.

For 118 plots, 70-year class: Cubic-foot volume per cent = 1.145 (basal area per cent) - 10.2.

There is nothing so disturbing, therefore, that we can not adopt the values of Tables 7 and 8 in the actual practice of yield application on an extensive scale. However, on small local areas the variation can be as wide as the limits prescribed by the standard errors of the curves.

QUALITY OF WOOD, AS WELL AS QUANTITY, AFFECTED BY STOCKING

The result of understocking upon the quality of the lumber produced is very important and is strikingly shown in Figure 8, but quality of wood in the tree is difficult to appraise. A study of quality growth not only must be distinct from the study of quantitative production, but it involves many additional technical difficulties and a large number of tests of the quality of logs or lumber produced in normal stands as well as in understocked stands. Observation shows that open-grown stands produce coarser-grained wood, more knots, and larger knots, but no qualitative data are available as to the actual quality differences between the product of normal stands and that of understocked stands. This is a question of prime importance and an attempt should be made to solve it, probably by cutting acres of different degrees of stocking, determining the grade or quality class of products yielded by actual conversion to merchantable units, and attempting to arrive at workable conclusions.

SUMMARY

Normal-yield tables are chiefly valuable as a standard for judging the future yield of existing forest stands. But it is well known that actual forests on extensive areas differ appreciably from the normal condition and by a degree which is not constant but is distinct for each separate area. Some forests may be very much understocked, some only slightly so, and some may even be overstocked. In order to use normal-yield tables to predict future yields of stands of all these degrees of stocking, it is necessary to know the fundamental relation between such stand factors as number of trees, basal area, and volume as affected by degree of stocking. Since degree of stocking must be defined in terms of volume, which is a value requiring much computation, it is desirable to have as simple a measure as possible for an index of stocking. This index would be used in the field for recognizing the degree of normality.

The relations found in this study between the normal yield table stand values and the stand values of actual forests concern only quantitative wood production. They do not take into consideration

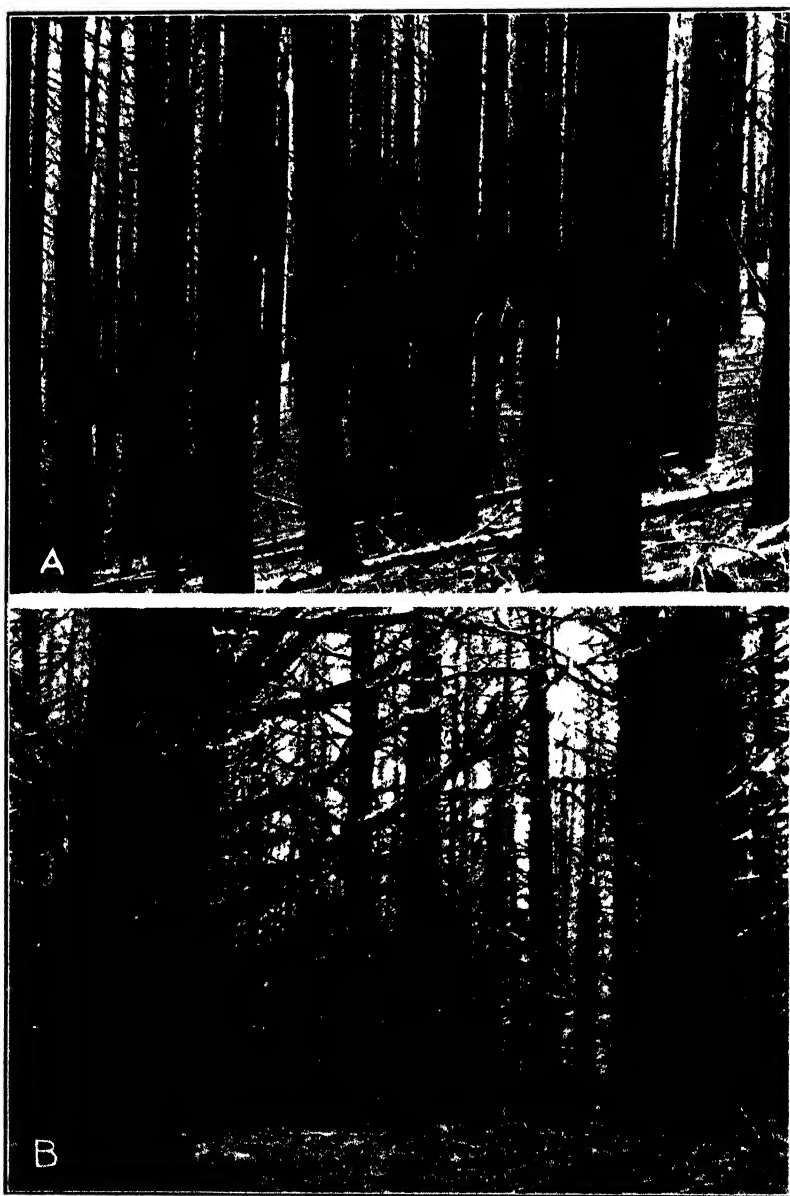


FIGURE 8.-- Single stands may show great variations in stocking. These two photographs were taken within a few hundred yards of each other. In a very widely spaced portion of the stand (B) the trees became overdeveloped and limby; where conditions were somewhat above normal, as in A, trees were of good form but had a much smaller average diameter than in the first area.

utilization of stands nor quality production, both most important but distinct phases of forest-yield predictions.

Some of the significant findings of this study may be summarized as follows:

To arrive at the real acreage of the type under consideration, the areas of all large openings and of other types must be mapped out and eliminated from consideration. Small holes and gaps are left in. In the Douglas fir canopy these small gaps amount on an average to 10 per cent of the total area. The most important cause of such gaps in this type is not fire, but the presence of gullies, creeks, and small hardwood patches.

Understocking is primarily attributable to the presence of small holes, and is only secondarily a factor of wider spacing.

Second-growth stands of Douglas fir, not including the major openings and gaps, approximate slightly better than 80 per cent of the normal-yield table values, this estimate being based on the results of strip surveys in 83 individual tracts.

Total number of trees is a very unsatisfactory index of total volume stocking; basal area is the best and most reliable index of volumes in cubic feet and board feet by the International rule. For board-foot volume by Scribner rule the number of trees 12 inches d. b. h. and larger is more reliable.

It is recommended that for short-term predictions basal area be the index for cubic-foot and International board-foot volume stocking, and that number of trees 12 inches and larger be the index for board-foot volume by Scribner rule. For long-term predictions basal area should be the index of stocking for all three units of volume.

Very little regarding the advance of an understocked stand to a more fully stocked, or normal, condition was learned from the analysis of these once-measured plots and strips, but periodic measurements of permanent plots indicate that this advance goes approximately at the rate of 4 per cent every decade for stands between 40 and 80 years of age.

The highest volumes are obtained on approximately 40 per cent slopes, referenced to horizontally measured acres, site for site. South and west aspects are apt to be quite variable in site. North and east aspects are the more favorable ones. Site apparently has no effect upon the average degree of stocking.

Sampling a tract by strips is considered more reliable than by plots, provided strip width be properly controlled. But the relation between pairs of stand factors, such as basal area, cubic-foot volume, and board-foot volume, are much alike whether determined by strip surveys or by sample plots.

STUDIES OF VACCINATION DURING CALFHOOD TO PREVENT BOVINE INFECTIOUS ABORTION¹

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HISTORICAL REVIEW

More than 30 years have elapsed since Bang announced his notable discovery with reference to the etiology of bovine infectious abortion. During this period an immense amount of work has been performed by different investigators in endeavors to improve methods of abortion prevention and control. In many cases the investigations reported have naturally dealt with immunization procedures. Data pertaining to this phase of the abortion problem have been contributed by Bang (1),³ McFadyean and Stockman (10), Zwick and his coworkers (12), Klimmer (8), Jensen (7), Smith and Little (11), Birch and Gilman (2), Hadley (4), Huddleson (6), Fitch and Boyd (3), Hart and Traum (5), Lubbehusen (9), and others. The substances employed as immunizing agents have included vaccines, prepared from living abortion bacteria of different degrees of virulence, bacterins consisting of suspensions of the abortion microorganism killed by subjection to heat and chemicals, serum with a high agglutinating titer for *Bacterium abortus*, sero vaccines, and sero bacterins.

Vaccines consisting of viable abortion bacteria, unless of unusually low virulence, have commonly been administered to unbred animals only. Bacterins and serums have been used both before and after breeding.

In some of the earlier investigations, before it was well understood that the abortion microorganism frequently invades and establishes itself in the udders of cows, the immunologist was principally concerned in testing the efficacy of biological substances in preventing the occurrence of abortions. Later studies have not only given consideration to this feature but have frequently been so planned and pursued as to reveal detrimental features as well as beneficial effects resulting from the procedures.

Many workers in this field believe that the degree of resistance to *Bacterium abortus* that can be imparted to susceptible stock by the use of abortion bacterin and serum is too meager in amount and of too brief duration to render such products of practical value in dealing with this disease. The available experimental data seem to justify this belief. Vaccines consisting of viable abortion bacteria, on the other hand, have yielded results that have been a source of considerable encouragement to both the investigator and the stockowner. Experiments with selected stock have plainly and repeatedly demonstrated that the vaccination of unbred, susceptible heifers about two

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² The writer thanks H. E. Smith for helpful assistance in making pregnancy examinations and agglutination tests and in otherwise aiding in the vaccination experiment.

³ Reference is made by number (italic) to Literature Cited, p. 682.

months before their breeding results in the development of an immunity that is both pronounced and lasting.

One of the arguments against vaccination has been that the administration of living suspensions of *Bacterium abortus* is followed by localization of the infection within the udder from which it may subsequently be eliminated for long periods of time. This characteristic has been noted with greater frequency after the vaccination of unbred cows with functioning udders than unbred heifers as they approach breeding age.

OBJECTS OF THE INVESTIGATION

The objects of the present investigation were twofold: (1) To determine whether a demonstrable and lasting degree of immunity could be engendered in bovines by their vaccination during calthood rather than at their approach to or attainment of maturity and (2) to determine the ability of calves to resist the prolonged or permanent establishment of *Bacterium abortus* infection in their bodies following the subcutaneous administration of the microorganism.

The original plan was to select for vaccination calves 3 to 4 months of age, for in the event the experiment yielded encouraging results it seemed possible that the method might be an appropriate one to employ at calf-branding time in infected herds maintained under range or semirange conditions.

EXPERIMENTAL ANIMALS

Twenty calves were procured for this work during May and June, 1925. They were assembled from herds in the vicinity of Washington, D. C., by a cattle dealer and delivered at the Bureau of Animal Industry Experiment Station, Bethesda, Md. It was impracticable to obtain information of value with reference to infectious abortion in the herds from which they were obtained. Some difficulty was experienced in procuring calves of the desired age, and it finally became necessary to accept animals that had the appearance of being from five to eight months in development. The calves seemed to be reasonably well bred, probably the progeny of grade stock. Their weights ranged from 275 to 400 pounds.

TABLE 1.—Results of the agglutination test for infectious abortion obtained from 20 calves purchased for vaccine experiment ^a

Calf No.	Results when quantity of blood serum (cubic centimeters) indicated was used						Calf No.	Results when quantity of blood serum (cubic centimeters) indicated was used					
	0.04	0.02	0.01	0.005	0.002	0.001		0.04	0.02	0.01	0.005	0.002	0.001
1263	—	—	—	—	—	—	1275	—	—	—	—	—	—
1264	—	—	—	—	—	—	1276	—	—	—	—	—	—
1265	—	—	—	—	—	—	1277	—	—	—	—	—	—
1266	—	—	—	—	—	—	1278	—	—	—	—	—	—
1267	—	—	—	—	—	—	1279	+	P	—	—	—	—
1268	—	—	—	—	—	—	1280	—	—	—	—	—	—
1269	—	—	—	—	—	—	1281	—	—	—	—	—	—
1272	+	+	P	—	—	—	1302	—	—	—	—	—	—
1273	—	—	—	—	—	—	1303	—	—	—	—	—	—
1274	—	—	—	—	—	—	1304	—	—	—	—	—	—

^a Key:

— = Pronounced agglutination.

P = Partial agglutination.

— = No agglutination.

An agglutination test for infectious abortion made soon after the calves were received resulted in the data shown in Table 1. Calf 1272 reacted in a dilution of 1 to 100 and calf 1279 in a dilution of 1 to 50. These animals consequently were not utilized in the experiment. Of the remaining 18, 12 were used as principals and 6 as controls.

MATERIALS AND METHODS

It was not definitely known what degree of virulence *Bacterium abortus* strains used in the preparation of vaccine should possess in order to be capable, when administered subcutaneously to virgin animals, of conferring an appreciable degree of immunity without at the same time causing a permanent infection of the udders. Hence, it was proposed to give this feature consideration.

Three different lots of vaccine were accordingly prepared. *Bacterium abortus* strains 8, 10, and 11 that had been propagated on artificial culture media for approximately eight years were used in the preparation of the lot of vaccine administered to calves 1263, 1264, 1265, 1266, 1267, and 1302. Strain 19 that had been isolated one and one-half years previously was used in the preparation of the vaccine administered to calves 1268, 1276, and 1277, and *Bact. abortus* strain 88, which was the third transfer of this isolation, was used in the preparation of the vaccine administered to calves 1273, 1274, and 1278.

Slants of 3 per cent glycerin agar in 18-mm. tubes were sown with the different *Bacterium abortus* strains three days before the vaccination of the animals. The suspensions were prepared immediately preceding their use. The 20 c. c. dose which each of the 12 principals received corresponded to the quantity of growth propagated on one tube of medium and represented in density a Gates nephelometer reading of about 1 cm. The vaccine was introduced subcutaneously at four different points on the sides of the neck and thorax in an endeavor to prevent severe swelling or abscess formation. Although no discharging abscesses developed at the points of inoculation it seems possible that the dose should have been reduced, for somewhat pronounced swellings appeared, where the injections were made, that persisted for several days. On the second and third days after the injections, moreover, the calves were sluggish and refused feed, and with one exception exhibited much elevated temperatures. Calf 1273, although showing no increase in temperature and but average local reactions, appeared to be the most seriously affected of the group. She refused feed for a week and was not inclined to mingle with the other calves. During this period she appeared to dislike to stand or to move about.

The temperatures of the animals before vaccination and for several days following are shown in Table 2. The somewhat elevated preinjection temperatures probably resulted partly from the excitement induced by catching and confining and partly from the high atmospheric temperature which prevailed at the time.

The vaccinated and control animals were kept in separate fields for about three months, when they were placed in the same stable and thereafter were run together. At monthly intervals blood samples were obtained and subjected to the agglutination test.

TABLE 2.—Temperatures of calves immediately preceding vaccination, September 15, 1925, and on five subsequent dates

Calf No	Preinjection temperature on Sept. 15, 1925	Postinjection temperature on—				
		Sept. 17, 1925	Sept. 18, 1925	Sept. 19, 1925	Sept. 21, 1925	Sept. 23, 1925
	° F.	° F.	° F.	° F.	° F.	° F.
1263	105.2	108.4	108.0	105.4	105.5	103.8
1264	104.0	107.2	107.2	104.8	104.1	101.4
1265	103.8	106.6	106.2	105.6	105.0	102.4
1266	104.0	107.8	106.8	104.8	105.0	102.4
1267	104.0	107.2	106.8	104.2	103.5	102.6
1268	103.0	107.2	106.9	103.8	104.1	102.5
1273	103.8	103.4	102.9	102.0	103.2	101.5
1274	103.6	106.8	106.0	104.8	105.4	103.0
1276	104.4	107.8	108.0	106.4	106.4	102.6
1277	104.6	107.4	107.0	105.4	106.5	102.0
1278	103.4	106.8	106.8	104.2	104.8	102.4
1302	106.0	108.2	107.6	106.6	106.4	104.8

When blood samples were being taken on December 10, 1925, approximately three months after the vaccination date, it was observed that the udder of heifer 1273, one of the animals which received the most recently isolated strain of *Bacterium abortus*, showed some distention. She was consequently isolated and milk serum obtained from her udder. The establishment of *Bact. abortus* infection in this animal seemed to be indicated by the fact that her blood-serum reactions had shown little tendency to recede in intensity. Failure was experienced, however, in demonstrating the presence of abortion bacteria in her udder secretions at this time, through guinea-pig-inoculation tests. A month later she was again placed with the remainder of the group.

Breeding of the heifers was begun during the middle of June, 1926. A vigorous 2-year-old bull that gave negative results to the agglutination test for infectious abortion was used for the entire group. Before all the animals had conceived one of the controls, No. 1281, became badly injured and, in consequence, had to be killed. One of the vaccinated animals, No. 1302, was found dead about a month after vaccination. An autopsy indicated that death was induced by internal hemorrhage, there being a rupture of the posterior vena cava a few inches from the heart. The rupture was associated with embolus formation. An effort was made to isolate *Bacterium abortus* from the embolus and the diseased walls of the vein as well as from other organs, but without success. There now remained in the experiment 16 heifers, 11 of which were principals and 5 controls.

On November 23, 1926, a fetus from a *Bacterium abortus* infected herd was available as exposure material for this group of animals, in all of which pregnancy had now been definitely diagnosed.

After small amounts of the stomach and intestinal contents had been sown on serum agar to determine the presence of *Bact. abortus* infection, the stomach content was drained into a flask. The intestinal content was washed out with saline. These materials were stored at refrigerator temperature. When the presence of *Bact. abortus* in large numbers was definitely established in these materials, they were strained through cheesecloth to remove the larger particles and made up to a 2,500 c. c. amount with physiological saline solution.

On November 26, 1926, each of the 16 pregnant heifers received 150 c. c. of the material by way of mouth.

A second fetus from another infected herd was available November 26, 1926. This fetus was handled in a manner similar to the one previously described. After the presence of *Bacterium abortus* in its digestive contents was established, 2,100 c. c. of a suspension of stomach and intestinal contents was used for a second exposure of the 16 heifers November 29, 1926. Each heifer at this time received 125 c. c. of the material by way of mouth.

When calving or aborting occurred, guinea pigs were inoculated with colostrum. Guinea pigs were also injected with placental emulsions or uterine exudate, unless it seemed evident that cultural results with aborted fetuses would reveal the presence of *Bacterium abortus* infection in the uteri of the aborting animals.

RESULTS OF FIRST PREGNANCIES

During their first pregnancies the 11 vaccinated animals produced 11 living calves. These, with one exception, continued to thrive for a year or longer, when they were utilized for other experimental purposes. The exception was the calf of heifer 1276, which died nine days after birth, and at autopsy showed severe inflammation of the intestines. A bipolar staining organism was isolated from the blood of this calf which may have caused its death. There was little evidence that its illness was in any way associated with *Bacterium abortus* infection.

Of the five controls, three, Nos. 1269, 1303, and 1304, aborted. *Bacterium abortus* was isolated from their fetuses by cultural methods and from samples of their colostrum through guinea-pig inoculations. Two of the controls, Nos. 1275 and 1280, produced vigorous calves, although heifer 1275, 47 days after calving, developed an agglutination reaction to *Bact. abortus* infection of a titer of 1 to 200, which was maintained for about one month. Efforts to isolate the abortion organism from her milk slightly subsequent to this period was attended with failure.

These two animals, Nos. 1275 and 1280, may have possessed unusual resistance to the infection, a supposition somewhat strengthened by the fact that on receiving large doses of *Bacterium abortus* suspensions at a later date when again pregnant, intravenously and through the teat canals, they produced, at the expiration of normal periods of pregnancy, living vigorous calves. Before the original intentional exposure both of these heifers had given suspicious reactions to the agglutination test on different occasions, a feature that may have accounted in a measure for their apparent resistance.

Although 11 living calves were produced by the 11 vaccinated animals during their first pregnancies the results were slightly less encouraging than this performance would imply. Although the agglutination reaction of heifer 1263 had returned to negative four months after vaccination and continued to remain negative or practically so for 15 months, it thereupon became markedly positive and remained thus for many months. The immunity which the vaccine afforded in her case was seemingly insufficient to enable her to resist the degree of *Bacterium abortus* exposure to which she was subsequently subjected. Although her calf was carried nearly the normal

period of gestation and seemed to exhibit normal vigor, the presence of *Bact. abortus* was demonstrated both in her placenta and colostrum.

Heifer 1273, in which *Bacterium abortus* seemed to have been established by the vaccine, although she produced a seemingly normal calf at the expiration of a 279-day gestation period, nevertheless eliminated the infection in her colostrum, as was demonstrated by guinea-pig inoculation results. Guinea pigs which were injected with placental emulsions, however, failed to acquire the disease.

Heifer 1263, as previously mentioned, received vaccine prepared from *Bacterium abortus* strains 8, 10, and 11 that had been propagated for about eight years on artificial culture media, whereas heifer 1273 received vaccine prepared from *Bact. abortus* strain 88, which was the third transfer of this strain. The results obtained with heifers 1263 and 1273 were, therefore, such as might possibly be anticipated in some cases when strains representing extremes in virulence are used for vaccine preparation.

TABLE 3.—Immunization results in animals vaccinated during calthood and later subjected to artificial exposure to *Bacterium abortus*-infected materials

TREATED WITH ABORTION VACCINE							
Animal No.	Date of vaccinations	Date of breeding	Date of exposure	Gestation period	Outcome of pregnancy	Results of uterine examinations for <i>Bacterium abortus</i>	Results of colostrum examinations for <i>Bacterium abortus</i>
	1925	1926	1926	Days			
1263	Sept. 15	Aug. 31 Oct. 11	Nov. 26 Nov. 29	277	Living calf	Positive	Positive
1264	do	June 22	Nov. 26 Nov. 29	284	do	Negative	Negative
1265	do	Aug. 27	Nov. 26 Nov. 29	279	do	do	Do.
1266	do	June 18	Nov. 26 Nov. 29	275	do	do	Do.
1267	do	June 21	Nov. 26 Nov. 29	268	do	do	Do.
1268	do	July 8	Nov. 26 Nov. 29	282	do	do	Do.
1273	do	June 24	Nov. 26 Nov. 29	279	do	do	Positive.
1274	do	(*)	Nov. 26 Nov. 29		do	do	Negative.
1276	do	July 1	Nov. 26 Nov. 29	277	do	do	Do.
1277	do	July 23	Nov. 26 Nov. 29	282	do	do	Do.
1278	do	June 26	Nov. 26 Nov. 29	268	do	do	Do.

CONTROLS

1269	(*)	June 20 July 8 Oct. 23	Nov. 26 Nov. 29	173	Abortion	Positive	Positive.
1275	(*)	June 20	Nov. 26 Nov. 29	279	Living calf	Negative	Negative.
1280	(*)	June 22	Nov. 26 Nov. 29	283	do	do	Do.
1303	(*)	July 3	Nov. 26 Nov. 29	215	Abortion	Positive	Positive.
1304	(*)	June 27	Nov. 26 Nov. 29	236	do	do	Do.

* No record.

† Not vaccinated.

Table 3 shows the date of vaccination, dates of breeding and of *Bacterium abortus* exposures, length of gestation periods, outcome of pregnancies, and results of uterine and colostrum examinations.

RESULTS OF SECOND PREGNANCIES

During the spring of 1928 the 11 vaccinated animals, without further treatment, were again bred to a bull giving negative results to the abortion test. The purpose was to gain some information as to the permanence of immunity engendered by calfhood vaccination. While it was realized that the ingestion exposure previously practiced complicated the problem of determining the lasting immunizing effects of vaccination alone, it was nevertheless believed that the existing conditions of exposure might not differ materially from the conditions encountered by vaccinated calves when in contact with infected herds.

Pregnancy examinations made during July and August, 1928, indicated that all 11 vaccinated heifers had conceived. Pregnancy was also determined in five other animals giving negative results to the agglutination test for the disease, and these animals were used as controls during the second pregnancies of the vaccinated group. This control group previously had been mated to an abortion-disease-free bull as indicated by the agglutination test. One of the controls, No. 1360, was a cow, the other four, Nos. 1419, 1425, 1426, and 1453, were heifers.

The *Bacterium abortus* exposure material used in this second period of the experiment was similar to that employed during the preceding pregnancies of the vaccinated animals. Two aborted fetuses were obtained from different herds on July 31 and August 6, 1928. Cultural work with these fetuses indicated that they were heavily infected with the abortion microorganism. The intestines were cut into small pieces with scissors and immersed and agitated in saline. The suspension was then passed through a wire sieve to remove the intestinal musculature. The suspension of intestinal contents was made up in each case to about 2,500 c. c. It was stored at a temperature of about 40° F. for several days, until cultural results were ascertained, before being used. To every experimental cow on each date of exposure was administered by way of mouth 100 c. c. of the material. Six days intervened between the times the cows were drenched, the first on August 3, 1928, the second on August 9. On the dates of drenching, subcutaneous inoculations of the exposure materials were made, into guinea pigs, to establish the infectiousness of the materials. These inoculations resulted in the development of marked lesions of abortion disease.

The results obtained after the second conception of the vaccinated animals did not differ markedly from those of the first. The 11 vaccinated animals produced 10 living calves, all of which made remarkably good growth.

Cow 1273 aborted at the expiration of a 176-day gestation period. *Bacterium abortus* could not be established as a causative factor, however, even though cultural examinations of the fetus were supplemented by guinea-pig inoculations of placental emulsions, colostrum, and emulsions of fetal stomach and intestinal contents. *Bacillus coli* was the predominating type of infection isolated from the

placenta. Cow 1263, from whose placenta and colostrum *Bact. abortus* was isolated at the previous pregnancy, although yielding positive colostrum results at this time, did not show *Bact. abortus* infection in the uterus, since guinea pigs inoculated with placental emulsions failed to acquire abortion disease. The uterine and colostrum results of the remaining nine vaccinated animals, as determined by guinea-pig inoculations at times of parturition, were negative.

Of the five control animals one heifer, No. 1426, aborted 218 days after her last breeding date. *Bacterium abortus* was isolated from her fetus by cultural means and from her colostrum by guinea-pig inoculations. Heifer 1425 expelled a weak calf on the two hundred fifty-eighth day of gestation. From the placenta and colostrum of this animal *Bact. abortus* was isolated. Heifer 1419 was in labor on the two hundred sixty-ninth day of gestation and required assistance in the removal of her calf, which was dead. A search for *Bact. abortus*, both culturally and through guinea-pig inoculations of placental and fetal materials, gave negative results. The serological reactions of this heifer supported the cultural and inoculation results.

Two of the five control animals produced vigorous calves.

TABLE 4.—Immunization results during second pregnancies of animals vaccinated during calfhood when again subjected to *Bacterium abortus*-ingestion exposure

TREATED BEFORE FIRST PREGNANCY WITH ABORTION VACCINE

Animal No	Date of breeding	Date of exposure	Gestation period	Outcome of pregnancy	Results of uterine examinations for <i>Bacterium abortus</i>	Results of colostrum examination for <i>Bacterium abortus</i>
	1928	1928	Days			
1263	Apr 11	{Aug 3 Aug 9	275	Living calf	Negative.	Positive
1264	Mar 27	{Aug 3 Aug 9	286	do	do	Negative.
1265	Apr 9	{Aug 3 Aug 9	286	do	do	Do
1266	Apr 4	{Aug 3 Aug 9	261	do	do	Do
1267	Mar 24	{Aug 3 Aug 9	279	do	do	Do
1268	Mar. 26	{Aug 3 Aug 9	280	do	do	Do.
1273	{Mar. 21 May 1	{Aug 3 Aug 9	176	Abortion.	do	Do.
1274	Mar 27	{Aug 3 Aug 9	282	Living calf	do	Do.
1276	Mar. 23	{Aug 3 Aug 9	281	do	do	Do.
1277	{Mar 22 Apr 21	{Aug 3 Aug 9	280	do	do	Do
1278	Apr. 2	{Aug 3 Aug 9	276	do	do	Do.

CONTROLS

1360	Feb. 6	{Aug. 3 Aug. 9	288	Living calf	Negative	Negative.
1419	Apr. 3	{Aug 3 Aug. 9	269	Dead calf	do	Do.
1425	Apr. 4	{Aug. 3 Aug. 9	258	Living calf	Positive	Positive.
1426	{Mar. 24 Apr. 14	{Aug. 3 Aug. 9	218	Abortion	do	Do.
1453	(*)	{Aug 3 Aug. 9		Living calf	Negative	Negative.

* No record.

Table 4 shows in condensed form the procedure followed and the results of the second phase of the calfhood-vaccination experiment.

Table 5 contains more complete records of the individual animals used in the experiment. In it are recorded the agglutination results that were obtained at varying intervals of time, the date of vaccination, dates of breeding, *Bacterium abortus* exposures, calvings, cultural determinations, and results of guinea-pig inoculations with colostrum, milk, and uterine products.

Those guinea pigs which were inoculated with the different materials and did not succumb to pneumonia, gastritis, or enteritis were killed in from five to eight weeks after inoculation, examined for the presence of lesions, and their blood subjected to the agglutination test for abortion disease. In some instances the spleens of the guinea pigs were cultured. This procedure was regarded as furnishing rather definite information as to the presence or absence of the infection in the inoculated pigs. The records of those guinea pigs that died soon after the injection, and that were not cultured or from which blood samples were not obtained for diagnostic determinations, are rarely entered in the table, because of the unreliability of the evidence which they furnished.

TABLE 5. Records of individual experiment animals ^a

VACCINATED HEIFER 1263

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used ^b										Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001		
May 21, 1925	-	-	-	-	-	-	-	-	-		
June 26, 1925	-	-	-	-	-	-	-	-	-		
July 29, 1925	-	-	-	-	-	-	-	-	-		
Sept. 2, 1925	-	-	-	-	-	-	-	-	-		
Oct. 4, 1925	+	+	+	+	+	+	+	P	-	Sept. 15, 1925, subcutaneous injection 20 c c abortion vaccine (<i>Bact. abortus</i> strains 8, 10, and 11).	
Nov. 10, 1925	+	+	+	+	-	-	-	-	-		
Dec. 10, 1925	+	+	+	-	-	-	-	-	-		
Jan. 15, 1926	-	-	-	-	-	-	-	-	-		
Feb. 15, 1926	-	-	-	-	-	-	-	-	-		
Mar. 16, 1926	S	-	-	-	-	-	-	-	-		
Apr. 22, 1926	-	-	-	-	-	-	-	-	-		
May 21, 1926	-	-	-	-	-	-	-	-	-		
June 14, 1926	P	-	-	-	-	-	-	-	-		
July 20, 1926	-	-	-	-	-	-	-	-	-		
Aug. 24, 1926	-	-	-	-	-	-	-	-	-		
Sept. 22, 1926	-	-	-	-	-	-	-	-	-	Aug. 31, 1926, bred.	
Nov. 23, 1926	-	-	-	-	-	-	-	-	-	Oct. 11, 1926, bred.	
Dec. 18, 1926	-	-	-	-	-	-	-	-	-	Nov. 26, 1926, received c 150 c c emulsion of fetal stomach and intestinal contents.	
Jan. 6, 1927	-	-	-	-	-	-	-	-	-	Nov. 29, 1926, received 125 c c emulsion of fetal stomach and intestinal contents.	
Jan. 22, 1927	-	-	-	-	-	-	-	-	-		
Feb. 4, 1927	-	-	-	-	-	-	-	-	-		
Mar. 7, 1927	-	-	-	-	-	-	-	-	-		
Mar. 21, 1927	-	-	-	-	-	-	-	-	-		
Apr. 4, 1927	-	-	-	-	-	-	-	-	-		
Apr. 19, 1927	+	+	+	+	P	-	-	-	-		
May 2, 1927	+	+	+	+	+	P	-	-	-		
May 17, 1927	+	+	+	+	+	+	+	-	-		
June 1, 1927	+	+	+	+	+	+	+	+	+		
June 16, 1927	+	+	+	+	+	+	+	+	+		
July 19, 1927	+	+	+	+	+	+	+	+	+		
Aug. 6, 1927	+	+	+	+	+	+	+	+	+		
Sept. 21, 1927	+	+	+	+	+	+	-	-	-	July 15, 1927, expelled a living calf (277 days), guinea pigs inoculated with emulsion of placenta and colostrum acquired abortion disease.	

^a Explanation of symbols: The plus sign indicates pronounced clumping of bacteria; P, partial clumping; S, slight trace of agglutination; minus sign (-), no evidence of clumping.

^b Figures at head of columns indicate cubic centimeters of blood serum. They represent approximate dilutions of 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, 1 to 1,000, 1 to 2,000, 1 to 5,000, and 1 to 10,000, respectively, with the amount of antigen (1 c. c.) used.

^c All exposures were made orally.

VACCINATED HEIFER 1263--Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used										Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001		
Oct. 20, 1927	+	+	+	+	+	-	-	-	-	Oct. 20, 1927, two guinea pigs inoculated with milk failed to acquire abortion disease.	
Nov. 19, 1927	+	+	+	+	+	P	-	-	-		
Jan. 25, 1928	+	+	+	+	+	+	+	P	P	Jan. 25, 1928, three guinea pigs inoculated with milk acquired abortion disease.	
June 6, 1928	+	+	+	+	+	+	-	-	-		
July 18, 1928	+	+	+	+	+	+	-	-	-	Apr. 11, 1928, bred.	
Aug. 24, 1928	+	+	+	+	+	+	-	-	-		
Sept. 19, 1928	P	P	+	+	+	+	-	-	-	Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.	
Oct. 12, 1928	+	+	+	+	+	-	-	-	-	Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.	
Oct. 27, 1928	+	+	+	+	+	P	-	-	-		
Nov. 16, 1928	+	+	+	+	+	P	-	-	-	Jan. 11, 1929, expelled a living calf (275 days); 5 guinea pigs inoculated with emulsion of placenta failed to acquire abortion disease, 5 inoculated with colostrum contracted abortion disease.	
Dec. 6, 1928	+	+	+	+	+	+	-	-	-		
Dec. 19, 1928	+	+	+	+	+	+	-	-	-		
Jan. 8, 1929	+	+	+	+	+	+	-	-	-		
Jan. 28, 1929	+	+	+	+	+	+	-	-	-		
Feb. 28, 1929	+	+	+	+	+	+	-	-	-		
Apr. 11, 1929	+	+	+	+	P	-	-	-	-		

VACCINATED HEIFER 1264

May 21, 1925	-	-	-	-	-	-	-	-
June 26, 1925	-	-	-	-	-	-	-	-
July 29, 1925	-	-	-	-	-	-	-	-
Sept. 2, 1925	-	-	-	-	-	-	-	-
Oct. 4, 1925	+	+	+	+	+	+	S	-
Nov. 10, 1925	+	+	+	+	+	+	+	-
Dec. 10, 1925	+	+	+	+	+	+	+	-
Jan. 15, 1926	P	-	-	-	-	-	-	-
Feb. 15, 1926	-	-	-	-	-	-	-	-
Mar. 16, 1926	+	+	+	+	+	+	+	-
Apr. 22, 1926	+	+	+	+	+	+	+	-
May 21, 1926	+	+	+	+	+	+	+	-
June 14, 1926	+	+	+	+	+	+	+	-
July 20, 1926	P	-	-	-	-	-	-	-
Aug. 24, 1926	P	-	-	-	-	-	-	-
Sept. 22, 1926	P	-	-	-	-	-	-	-
Nov. 23, 1926	P	-	-	-	-	-	-	-
Dec. 18, 1926	S	-	-	-	-	-	-	-
Jan. 6, 1927	P	-	-	-	-	-	-	-
Jan. 22, 1927	S	-	-	-	-	-	-	-
Feb. 4, 1927	P	-	-	-	-	-	-	-
Mar. 7, 1927	P	-	-	-	-	-	-	-
Mar. 21, 1927	-	-	-	-	-	-	-	-
Apr. 4, 1927	-	-	-	-	-	-	-	-
Apr. 19, 1927	-	-	-	-	-	-	-	-
May 2, 1927	-	-	-	-	-	-	-	-
May 17, 1927	P	-	-	-	-	-	-	-
June 16, 1927	-	-	-	-	-	-	-	-
July 19, 1927	-	-	-	-	-	-	-	-
Aug. 6, 1927	-	-	-	-	-	-	-	-
Sept. 21, 1927	-	-	-	-	-	-	-	-
Oct. 20, 1927	-	-	-	-	-	-	-	-
Nov. 19, 1927	-	-	-	-	-	-	-	-
Jan. 25, 1928	-	-	-	-	-	-	-	-
June 6, 1928	-	-	-	-	-	-	-	-
July 18, 1928	P	-	-	-	-	-	-	-
Aug. 24, 1928	P	-	-	-	-	-	-	-
Sept. 19, 1928	+	-	-	-	-	-	-	-
Oct. 12, 1928	-	-	-	-	-	-	-	-
Oct. 27, 1928	+	S	-	-	-	-	-	-
Nov. 16, 1928	-	-	-	-	-	-	-	-
Dec. 6, 1928	S	-	-	-	-	-	-	-
Dec. 19, 1928	-	-	-	-	-	-	-	-
Jan. 8, 1929	-	-	-	-	-	-	-	-
Jan. 28, 1929	-	-	-	-	-	-	-	-

Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact abortus* strains 8, 10, and 11).

June 22, 1926, bred.

Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.

Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.

Apr. 2, 1927, expelled a living calf (284 days). 4 guinea pigs inoculated with uterine material and 4 with colostrum failed to develop abortion disease.

June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.

Mar. 27, 1928, bred.

Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.

Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.

Jan. 7, 1929, expelled a living calf (286 days); 4 guinea pigs inoculated with an emulsion of placenta and 6 with colostrum failed to acquire abortion disease.

[illegible]

TABLE 5.—Records of individual experiment animals—Continued

VACCINATED HEIFER 1268—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used										Remarks
	0 04	0 02	0 01	0.005	0.002	0.001	0.0005	0.0002	0 0001		
Oct 12, 1928	+	—	—	—	—	—	—	—	—	Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.	
Oct 27, 1928	+	P	—	—	—	—	—	—	—		
Nov. 16, 1928	+	S	—	—	—	—	—	—	—		
Dec. 6, 1928	S	—	—	—	—	—	—	—	—	Dec. 31, 1928, expelled a living calf (280 days); 6 guinea pigs inoculated with uterine material and 6 with colostrum failed to contract abortion disease.	
Dec 19, 1928	P	—	—	—	—	—	—	—	—		
Jan. 8, 1929	+	P	—	—	—	—	—	—	—		
Jan 28, 1929	+	—	—	—	—	—	—	—	—		
Feb 28, 1929	+	P	—	—	—	—	—	—	—		
Apr. 11, 1929	+	P	—	—	—	—	—	—	—		

VACCINATED HEIFER 1273

May 21, 1925	—	—	—	—	—	—	—	—	—	Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (<i>Bact. abortus</i> strain 88).
June 26, 1925	—	—	—	—	—	—	—	—	—	
July 29, 1925	—	—	—	—	—	—	—	—	—	
Sept. 2, 1925	—	—	—	—	—	—	—	—	—	
Oct. 4, 1925	+	+	+	+	+	+	+	+	+	
Nov. 10, 1925	+	+	+	+	+	+	+	+	+	
Dec. 10, 1925	+	+	+	+	+	+	+	+	+	
Jan. 15, 1926	+	+	+	+	+	+	+	+	+	
Feb. 15, 1926	+	+	+	+	+	+	+	+	+	
Mar. 16, 1926	+	+	+	+	+	+	+	+	+	
Apr. 22, 1926	+	+	+	+	+	+	+	+	+	June 24, 1926, bred.
May 21, 1926	+	+	+	+	+	+	+	+	+	
June 14, 1926	+	+	+	+	+	+	+	+	+	
July 20, 1926	+	+	+	+	P	—	—	—	—	
Aug. 24, 1926	+	+	+	+	+	—	—	—	—	
Sept. 22, 1926	+	+	+	S	—	—	—	—	—	
Oct. 19, 1926	+	+	+	P	—	—	—	—	—	
Nov. 23, 1926	+	+	+	+	+	—	—	—	—	
Dec. 18, 1926	+	+	+	+	—	—	—	—	—	
Jan. 6, 1927	+	+	+	+	+	—	—	—	—	Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents. Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	+	+	+	+	+	P	—	—	—	
Feb. 4, 1927	+	+	+	+	+	+	—	—	—	
Feb. 19, 1927	+	+	+	+	+	+	—	—	—	
Mar. 7, 1927	+	+	+	+	+	P	—	—	—	
Mar. 21, 1927	+	+	+	+	+	+	—	—	—	
Apr. 4, 1927	+	+	+	+	+	—	—	—	—	
Apr. 19, 1927	+	+	+	+	P	—	—	—	—	
May 2, 1927	+	+	+	+	+	—	—	—	—	
May 17, 1927	+	+	+	+	+	—	—	—	—	Mar. 30, 1927, expelled a living calf (279 days), 4 guinea pigs inoculated with uterine material failed to contract abortion disease, 4 inoculated with colostrum acquired abortion disease. May 17, 1927, 3 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
June 1, 1927	+	+	+	+	P	—	—	—	—	
June 16, 1927	+	+	+	+	+	—	—	—	—	
July 19, 1927	+	+	+	+	+	—	—	—	—	
Aug. 6, 1927	+	+	+	+	+	—	—	—	—	
Sept. 21, 1927	+	+	S	S	—	—	—	—	—	
Oct. 20, 1927	+	+	+	—	—	—	—	—	—	
Nov. 19, 1927	+	+	+	P	—	—	—	—	—	
Jan. 25, 1928	+	+	+	+	—	—	—	—	—	Oct. 20, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease. Jan. 25, 1928, 3 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease. Mar. 21, 1928, bred. May 1, 1928, bred. Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents. Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents. Oct. 24, 1928, aborted (176 days); cultural results with fetus negative for <i>Bact. abortus</i> ; 4 guinea pigs inoculated with placental emulsion, 4 with stomach and intestinal contents of fetus, and 6 with colostrum failed to contract abortion disease.
June 6, 1928	+	+	P	—	—	—	—	—	—	
July 18, 1928	+	+	+	P	—	—	—	—	—	
Aug. 24, 1928	+	+	+	—	—	—	—	—	—	
Sept. 19, 1928	+	+	+	—	—	—	—	—	—	
Oct. 12, 1928	+	+	+	—	—	—	—	—	—	
Oct. 27, 1928	+	+	+	P	—	—	—	—	—	
Nov. 16, 1928	+	+	+	P	—	—	—	—	—	
Dec. 6, 1928	+	+	+	P	—	—	—	—	—	
Dec. 19, 1928	+	+	+	+	—	—	—	—	—	
Jan. 8, 1929	+	+	+	P	—	—	—	—	—	
Jan. 28, 1929	+	+	+	P	—	—	—	—	—	
Feb. 28, 1929	+	+	+	+	—	—	—	—	—	
Apr. 11, 1929	+	+	+	+	—	—	—	—	—	

TABLE 5.—Records of individual experiment animals—Continued

VACCINATED HEIFER 1271

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0 04	0 02	0 01	0 005	0 002	0 001	0 0005	0 0002	0 0001	
May 21, 1925	-	-	-	-	-	-	-	-	-	Sept 15, 1925, subcutaneous injection 20 c c abortion vaccine (<i>Bact abortus</i> strain 88)
June 26, 1925	-	-	-	-	-	-	-	-	-	
July 29, 1925	-	-	-	-	-	-	-	-	-	
Sept 2, 1925	-	-	-	-	-	-	-	-	-	
Oct 4, 1925	+	+	+	+	+	+	-	-	-	
Nov 10, 1925	+	+	+	+	-	-	-	-	-	
Dec 10, 1925	+	+	-	-	-	-	-	-	-	No breeding record
Jan 15, 1926	P	S	-	-	-	-	-	-	-	
Feb 15, 1926	P	-	-	-	-	-	-	-	-	
Mar 16, 1926	+	+	-	-	-	-	-	-	-	
Apr. 22, 1926	-	-	-	-	-	-	-	-	-	
May 21, 1926	-	-	-	-	-	-	-	-	-	
June 14, 1926	+	-	-	-	-	-	-	-	-	
July 20, 1926	P	-	-	-	-	-	-	-	-	
Aug 24, 1926	-	-	-	-	-	-	-	-	-	Nov 26, 1926, received 150 c c emulsion of fetal stomach and intestinal contents.
Sept 22, 1926	S	-	-	-	-	-	-	-	-	
Oct 19, 1926	-	-	-	-	-	-	-	-	-	Nov 29, 1926, received 125 c c emulsion of fetal stomach and intestinal contents.
Nov 23, 1926	-	-	-	-	-	-	-	-	-	
Dec 18, 1926	-	-	-	-	-	-	-	-	-	Aug 18, 1927, expelled a living calf. 2 guinea pigs inoculated with placental emulsion and 2 with colostrum failed to contract abortion disease. Mar 27, 1928, bred
Jan 6, 1927	-	-	-	-	-	-	-	-	-	
Jan 22, 1927	-	-	-	-	-	-	-	-	-	
Feb 4, 1927	-	-	-	-	-	-	-	-	-	
Feb 19, 1927	-	-	-	-	-	-	-	-	-	
Mar 7, 1927	-	-	-	-	-	-	-	-	-	
Mar. 21, 1927	-	-	-	-	-	-	-	-	-	
Apr 4, 1927	-	-	-	-	-	-	-	-	-	
Apr 19, 1927	-	-	-	-	-	-	-	-	-	
May 2, 1927	-	-	-	-	-	-	-	-	-	
May 17, 1927	-	-	-	-	-	-	-	-	-	
June 1, 1927	-	-	-	-	-	-	-	-	-	
June 16, 1927	-	-	-	-	-	-	-	-	-	
July 19, 1927	-	-	-	-	-	-	-	-	-	
Aug 6, 1927	-	-	-	-	-	-	-	-	-	
Sept 21, 1927	-	-	-	-	-	-	-	-	-	
Oct 20, 1927	-	-	-	-	-	-	-	-	-	
Nov 19, 1927	-	-	-	-	-	-	-	-	-	
Jan 25, 1928	-	-	-	-	-	-	-	-	-	Aug 3, 1928, received 100 c c emulsion of fetal intestinal contents.
June 6, 1928	-	-	-	-	-	-	-	-	-	
July 18, 1928	-	-	-	-	-	-	-	-	-	Aug 9, 1928, received 100 c c emulsion of fetal intestinal contents
Aug 24, 1928	-	-	-	-	-	-	-	-	-	
Sept 19, 1928	-	-	-	-	-	-	-	-	-	Jan 3, 1929, expelled a living calf (282 days), 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to contract abortion disease.
Oct 12, 1928	-	-	-	-	-	-	-	-	-	
Oct 27, 1928	-	-	-	-	-	-	-	-	-	
Nov. 16, 1928	-	-	-	-	-	-	-	-	-	
Dec 6, 1928	-	-	-	-	-	-	-	-	-	
Dec 19, 1928	-	-	-	-	-	-	-	-	-	
Jan 8, 1929	-	-	-	-	-	-	-	-	-	
Jan 28, 1929	-	-	-	-	-	-	-	-	-	
Feb. 28, 1929	-	-	-	-	-	-	-	-	-	
Apr 11, 1929	-	-	-	-	-	-	-	-	-	

VACCINATED HEIFER 1276

[illegible]

May 21, 1925	-	-	-	-	-	-	-
June 26, 1925	-	-	-	-	-	-	-
July 20, 1925	-	-	-	-	-	-	-
Sept. 2, 1925	-	-	-	-	-	-	-
Oct. 4, 1925	+	+	+	+	+	+	P
Nov. 11, 1925	+	+	+	+	+	+	-
Dec. 10, 1925	+	+	+	+	+	+	-
Jan. 15, 1926	+	+	+	+	+	+	-
Feb. 15, 1926	+	+	+	z	+	+	-
Mar. 16, 1926	+	+	+	P	+	+	-
Apr. 22, 1926	+	+	+	-	+	+	-
May 21, 1926	+	+	+	z	+	+	-
June 14, 1926	+	+	+	-	+	+	-
July 20, 1926	+	+	+	-	+	+	-
Aug. 24, 1926	+	+	+	-	+	+	-
Sept. 22, 1926	+	+	+	-	+	+	-
Oct. 19, 1926	+	+	+	-	+	+	-
Nov. 23, 1926	+	+	P	-	+	+	-
Dec. 18, 1927	+	+	P	-	+	+	-
Jan. 6, 1927	+	+	P	-	+	+	-
Jan. 22, 1927	+	+	+	-	+	+	-
Feb. 4, 1927	+	+	+	-	+	+	-
Feb. 19, 1927	+	+	+	-	+	+	-
Mar. 7, 1927	+	+	+	-	+	+	-
Mar. 21, 1927	+	+	z	-	+	+	-
Apr. 4, 1927	+	+	+	-	+	+	-
Apr. 19, 1927	+	+	+	-	+	+	-
May 2, 1927	+	+	P	-	+	+	-
May 17, 1927	+	+	+	-	+	+	-
June 1, 1927	+	+	-	-	+	+	-
June 16, 1927	+	+	-	-	+	+	-
July 19, 1927	+	-	-	-	+	+	-
Aug. 6, 1927	+	-	-	-	+	+	-
Sept. 21, 1927	+	-	-	-	+	+	-
Oct. 20, 1927	+	-	-	-	+	+	-
Nov. 19, 1927	+	-	-	-	+	+	-

Sept. 15, 1925, subcutaneous injection 20 c.c. abortion vaccine (*Bact abortus* strain 19).

July 23, 1926, bred.

Nov. 26, 1926, received 150 c.c. emulsion of fetal stomach and intestinal contents.

Nov. 29, 1926, received 125 c.c. emulsion of fetal stomach and intestinal contents.

May 1, 1927, expelled a living calf (282 days); 4 guinea pigs inoculated with placental emulsion and 4 with colostrum failed to contract abortion disease.

June 15, 1927, 3 guinea pigs inoculated with milk failed to contract abortion disease.

TABLE 5.—Records of individual experiment animals.—Continued
VACCINATED HEIFER 1277—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Jan. 25, 1928	+	P	—	—	—	—	—	—	—	Mar. 22, 1928, bred. Apr. 21, 1928, bred.
June 6, 1928	+	S	—	—	—	—	—	—	—	
July 18, 1928	+	P	—	—	—	—	—	—	—	Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents
Aug. 24, 1928	+	P	—	—	—	—	—	—	—	
Sept. 19, 1928	+	+	—	—	—	—	—	—	—	Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents
Oct. 12, 1928	+	S	—	—	—	—	—	—	—	
Oct. 27, 1928	+	P	—	—	—	—	—	—	—	Jan. 25, 1928, expelled a living calf (280 days); 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to contract abortion dis- ease.
Nov. 16, 1928	+	P	—	—	—	—	—	—	—	
Dec. 6, 1928	+	+	—	—	—	—	—	—	—	
Dec. 19, 1928	+	P	—	—	—	—	—	—	—	
Jan. 8, 1929	+	+	—	—	—	—	—	—	—	
Jan. 24, 1929	+	P	—	—	—	—	—	—	—	
Feb. 28, 1929	+	P	—	—	—	—	—	—	—	
Apr. 11, 1929	+	S	—	—	—	—	—	—	—	

VACCINATED HEIFER 1278

May 21, 1925	—	—	—	—	—	—	—	—	—	Sept. 15, 1925, subcutaneous in- jection 20 c. c. abortion vac- cine (<i>Bact. abortus</i> strain 88).
June 25, 1925	—	—	—	—	—	—	—	—	—	
July 29, 1925	—	—	—	—	—	—	—	—	—	June 26, 1926, bred.
Sept. 2, 1925	—	—	—	—	—	—	—	—	—	
Oct. 4, 1925	+	+	+	+	+	+	+	+	P	
Nov. 10, 1925	+	+	+	+	+	+	+	+	+	
Dec. 10, 1925	+	+	+	+	+	+	+	+	+	
Jan. 15, 1926	+	+	+	+	+	+	+	+	+	
Feb. 15, 1926	+	P	—	—	—	—	—	—	—	
Mar. 16, 1926	+	P	—	—	—	—	—	—	—	
Apr. 22, 1926	P	S	—	—	—	—	—	—	—	
May 21, 1926	+	P	—	—	—	—	—	—	—	
June 14, 1926	+	P	—	—	—	—	—	—	—	Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents. Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
July 20, 1926	+	P	—	—	—	—	—	—	—	
Aug. 21, 1926	+	P	—	—	—	—	—	—	—	
Sept. 22, 1926	+	+	—	—	—	—	—	—	—	
Oct. 19, 1926	+	P	—	—	—	—	—	—	—	
Nov. 23, 1926	+	P	—	—	—	—	—	—	—	
Dec. 18, 1926	+	P	—	—	—	—	—	—	—	
Jan. 6, 1927	+	P	—	—	—	—	—	—	—	
Jan. 22, 1927	P	P	—	—	—	—	—	—	—	
Feb. 4, 1927	+	P	—	—	—	—	—	—	—	Mar. 21, 1927, expelled a living calf (268 days); 2 guinea pigs inoculated with uterine mater- ial and 4 with colostrum failed to acquire abortion dis- ease. June 15, 1927, 2 guinea pigs in- oculated with a composite sample of milk failed to con- tract abortion disease.
Feb. 19, 1927	+	P	—	—	—	—	—	—	—	
Mar. 7, 1927	+	S	—	—	—	—	—	—	—	
Mar. 21, 1927	+	S	—	—	—	—	—	—	—	
Apr. 4, 1927	+	+	—	—	—	—	—	—	—	
Apr. 19, 1927	+	—	—	—	—	—	—	—	—	
May 2, 1927	P	—	—	—	—	—	—	—	—	
May 17, 1927	+	—	—	—	—	—	—	—	—	
June 1, 1927	+	—	—	—	—	—	—	—	—	
June 16, 1927	+	—	—	—	—	—	—	—	—	
July 19, 1927	+	S	—	—	—	—	—	—	—	Apr. 2, 1928, bred
Aug. 6, 1927	+	—	—	—	—	—	—	—	—	
Sept. 21, 1927	+	—	—	—	—	—	—	—	—	
Oct. 20, 1927	—	—	—	—	—	—	—	—	—	
Nov. 19, 1927	P	—	—	—	—	—	—	—	—	
Jan. 25, 1928	+	—	—	—	—	—	—	—	—	
June 6, 1928	P	—	—	—	—	—	—	—	—	
July 18, 1928	P	—	—	—	—	—	—	—	—	
Aug. 24, 1928	+	S	—	—	—	—	—	—	—	
Sept. 19, 1928	+	—	—	—	—	—	—	—	—	
Oct. 12, 1928	+	—	—	—	—	—	—	—	—	Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	—	—	—	—	—	—	—	—	—	
Nov. 16, 1928	P	—	—	—	—	—	—	—	—	Jan. 3, 1929, expelled a living calf (276 days); 6 guinea pigs inoculated with placental emulsion and 6 with colos- trum failed to contract abor- tion disease.
Dec. 6, 1928	—	—	—	—	—	—	—	—	—	
Dec. 19, 1928	P	—	—	—	—	—	—	—	—	
Jan. 8, 1929	P	—	—	—	—	—	—	—	—	
Jan. 28, 1929	—	—	—	—	—	—	—	—	—	
Feb. 28, 1929	P	—	—	—	—	—	—	—	—	
Apr. 11, 1929	P	—	—	—	—	—	—	—	—	

CONTROL HEIFER 1269

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
May 21, 1925	-	-	-	-	-	-	-	-	-	
June 26, 1925	-	-	-	-	-	-	-	-	-	
July 29, 1925	-	-	-	-	-	-	-	-	-	
Sept. 2, 1925	-	-	-	-	-	-	-	-	-	
Oct. 4, 1925	-	-	-	-	-	-	-	-	-	
Nov. 10, 1925	-	-	-	-	-	-	-	-	-	
Dec. 10, 1925	-	-	-	-	-	-	-	-	-	
Jan. 15, 1926	-	-	-	-	-	-	-	-	-	
Feb. 15, 1926	-	-	-	-	-	-	-	-	-	
Mar. 16, 1926	-	-	-	-	-	-	-	-	-	
Apr. 22, 1926	-	-	-	-	-	-	-	-	-	
May 21, 1926	-	-	-	-	-	-	-	-	-	
June 14, 1926	-	-	-	-	-	-	-	-	-	June 20, 1926, bred.
July 20, 1926	-	-	-	-	-	-	-	-	-	July 8, 1926, bred.
Aug. 21, 1926	-	-	-	-	-	-	-	-	-	
Sept. 22, 1926	-	-	-	-	-	-	-	-	-	
Oct. 19, 1926	-	-	-	-	-	-	-	-	-	Oct. 23, 1926, bred.
Nov. 23, 1926	-	-	-	-	-	-	-	-	-	Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	P	-	-	-	-	-	-	-	Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 6, 1927	P	-	-	-	-	-	-	-	-	
Jan. 22, 1927	S	-	-	-	-	-	-	-	-	
Feb. 4, 1927	+	+	-	-	-	-	-	-	-	
Feb. 19, 1927	+	+	S	-	-	-	-	-	-	
Mar. 21, 1927	+	+	+	+	+	+	-	-	-	
Apr. 4, 1927	+	+	+	+	+	P	-	-	-	
Apr. 19, 1927	+	+	+	P	P	-	-	-	-	
May 2, 1927	+	+	+	+	+	P	-	-	-	Apr. 14, 1927, expelled a dead fetus (173 days). <i>Bact abortus</i> isolated from fetus and 4 guinea pigs inoculated with colostrum acquired abortion disease.
May 17, 1927	+	+	+	+	+	-	-	-	-	
June 1, 1927	+	+	+	+	-	-	-	-	-	June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk contracted abortion disease.
June 16, 1927	+	+	+	+	P	P	-	-	-	
July 19, 1927	+	+	+	+	-	-	-	-	-	Oct. 20, 1927, 2 guinea pigs in- oculated with a composite sample of milk acquired abor- tion disease.
Aug. 6, 1927	+	+	+	+	-	-	-	-	-	
Sept. 21, 1927	+	+	+	+	-	-	-	-	-	
Oct. 20, 1927	+	+	+	-	-	-	-	-	-	
Nov. 19, 1927	+	+	+	-	-	-	-	-	-	
Jan. 25, 1928	+	+	+	-	-	-	-	-	-	Jan. 25, 1928, 2 guinea pigs in- oculated with a composite sample of milk acquired abor- tion disease.
Apr. 12, 1928	+	+	+	-	-	-	-	-	-	

CONTROL HEIFER 1275

[illegible]

TABLE 5.—Records of individual experiment animals—Continued

CONTROL HEIFER 1303—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used										Remarks
	0 04	0 02	0 01	0 005	0.002	0.001	0.0005	0.0002	0 0001		
May 21, 1926	-	-	-	-	-	-	-	-	-		
June 14, 1926	-	-	-	-	-	-	-	-	-		
July 20, 1926	-	-	-	-	-	-	-	-	-	July 3, 1926, bred.	
Aug. 24, 1926	-	-	-	-	-	-	-	-	-		
Sept. 22, 1926	-	-	-	-	-	-	-	-	-		
Oct. 19, 1926	-	-	-	-	-	-	-	-	-		
Nov. 23, 1926	-	-	-	-	-	-	-	-	-	Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents	
Dec. 18, 1926	-	-	-	-	-	-	-	-	-	Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents	
Jan. 6, 1927	-	-	-	-	-	-	-	-	-		
Jan. 22, 1927	-	-	-	-	-	-	-	-	-	Feb. 3, 1927, expelled a dead fetus (215 days); <i>Bact abortus</i> isolated from fetus; 4 guinea pigs inoculated with colos- trum contracted abortion disease	
Feb. 4, 1927	+	-	-	-	-	-	-	-	-		
Feb. 19, 1927	P	-	-	-	-	-	-	-	-		
Mar. 7, 1927	+	+	-	-	-	-	-	-	-		
Mar. 21, 1927	+	+	+	+	+	-	-	-	-		
Apr. 4, 1927	+	+	-	-	+	-	-	-	-		
Apr. 19, 1927	+	+	+	+	+	P	-	-	-		
May 2, 1927	+	+	+	+	+	-	-	-	-		
May 17, 1927	+	+	+	+	-	-	-	-	-		
June 1, 1927	+	+	+	+	-	-	-	-	-		
June 16, 1927	+	+	+	+	-	-	-	-	-	June 15, 1927, 2 guinea pigs inoc- ulated with a composite sam- ple of milk failed to contract abortion disease	
July 19, 1927	+	+	+	+	-	-	-	-	-		
Aug. 6, 1927	+	+	+	+	+	+	-	-	-		
Sept. 21, 1927	+	+	+	+	+	+	-	-	-		
Oct. 20, 1927	+	+	+	+	+	+	+	-	-	Oct. 20, 1927, 2 guinea pigs inoc- ulated with a composite sample of milk contracted abortion disease.	
Nov. 19, 1927	+	+	+	+	+	+	-	-	-		
Jan. 25, 1928	+	+	+	+	+	+	+	+	-		

CONTROL HEIFER 1304

May 21, 1925	—	—	—	—	—	—	—	—	—	
June 26, 1925	—	—	—	—	—	—	—	—	—	
July 29, 1925	—	—	—	—	—	—	—	—	—	
Sept. 2, 1925	—	—	—	—	—	—	—	—	—	
Oct. 4, 1925	—	—	—	—	—	—	—	—	—	
Nov. 10, 1925	—	—	—	—	—	—	—	—	—	
Dec. 10, 1925	—	—	—	—	—	—	—	—	—	
Jan. 15, 1926	—	—	—	—	—	—	—	—	—	
Feb. 15, 1926	—	—	—	—	—	—	—	—	—	
Mar. 16, 1926	—	—	—	—	—	—	—	—	—	
Apr. 22, 1926	—	—	—	—	—	—	—	—	—	
May 21, 1926	—	—	—	—	—	—	—	—	—	
June 14, 1926	—	—	—	—	—	—	—	—	—	June 27, 1926, bred
July 20, 1926	—	—	—	—	—	—	—	—	—	
Aug. 24, 1926	—	—	—	—	—	—	—	—	—	
Sept. 22, 1926	—	—	—	—	—	—	—	—	—	
Oct. 19, 1926	—	—	—	—	—	—	—	—	—	
Nov. 23, 1926	—	—	—	—	—	—	—	—	—	Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents
Dec. 18, 1926	+	+	—	—	—	—	—	—	—	Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents
Jan. 6, 1927	+	+	P	—	—	—	—	—	—	Feb. 18, 1927, expelled a dead fetus (236 days); <i>Bact abortus</i> isolated from fetus; 5 guinea pigs inoculated with colos- trum contracted abortion disease
Jan. 22, 1927	+	+	+	+	S	—	—	—	—	June 15, 1927, 2 guinea pigs inoc- ulated with a composite sam- ple of milk contracted abortion disease.
Feb. 4, 1927	+	+	+	+	+	+	—	—	—	
Feb. 19, 1927	+	+	+	+	+	+	+	+	—	Oct. 20, 1927, 2 guinea pigs inoc- ulated with a composite sample of milk contracted abortion disease.
May 2, 1927	+	+	+	+	+	+	+	+	+	
May 17, 1927	+	+	+	+	+	+	+	+	+	
June 1, 1927	+	+	+	+	+	+	+	+	+	
June 16, 1927	+	+	+	+	+	+	+	+	+	
July 19, 1927	+	+	+	+	+	+	+	+	—	
Aug. 6, 1927	+	+	+	+	+	+	+	—	—	
Sept. 21, 1927	+	+	+	+	P	—	—	—	—	
Oct. 20, 1927	+	+	+	+	+	+	—	—	—	
Nov. 19, 1927	+	+	+	+	+	+	—	—	—	
Jan. 25, 1928	+	+	+	+	+	+	—	—	—	

TABLE 5.—Records of individual experiment animals—Continued

CONTROL COW 1300										
Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									
	0 01	0 02	0 01	0 005	0 002	0 001	0 0005	0 0002	0 0001	Remarks
June 2, 1928	—	—	—	—	—	—	—	—	—	Feb 6, 1928, bred
July 18, 1928	—	—	—	—	—	—	—	—	—	
Aug. 24, 1928	—	—	—	—	—	—	—	—	—	Aug 3, 1928, received 100 c c emulsion of fetal intestinal contents
Sept 19, 1928	—	—	—	—	—	—	—	—	—	Aug 9, 1928, received 100 c c emulsion of fetal intestinal contents
Oct 12, 1928	—	—	—	—	—	—	—	—	—	Nov 21, 1928, expelled a living calf (288 days), 6 guinea pigs inoculated with placental material and 5 with colostrum failed to contract abortion disease
Oct 27, 1928	—	—	—	—	—	—	—	—	—	
Nov 16, 1928	—	—	—	—	—	—	—	—	—	
Dec 6, 1928	—	—	—	—	—	—	—	—	—	
Dec 19, 1928	—	—	—	—	—	—	—	—	—	
Jan 8, 1929	—	—	—	—	—	—	—	—	—	
Jan 28, 1929	—	—	—	—	—	—	—	—	—	
CONTROL HEIFER 1419										
June 2, 1928	—	—	—	—	—	—	—	—	—	Apr 3, 1928, bred
June 6, 1928	—	—	—	—	—	—	—	—	—	
July 18, 1928	—	—	—	—	—	—	—	—	—	Aug 3, 1928, received 100 c c emulsion of fetal intestinal contents
Aug 24, 1928	—	—	—	—	—	—	—	—	—	Aug 9, 1928, received 100 c c emulsion of fetal intestinal contents
Sept 19, 1928	—	—	—	—	—	—	—	—	—	
Oct 12, 1928	—	—	—	—	—	—	—	—	—	
Oct 27, 1928	—	—	—	—	—	—	—	—	—	
Nov 16, 1928	—	—	—	—	—	—	—	—	—	
Dec 6, 1928	—	—	—	—	—	—	—	—	—	
Dec 19, 1928	—	—	—	—	—	—	—	—	—	
Jan 8, 1929	—	—	—	—	—	—	—	—	—	
Jan 28, 1929	—	—	—	—	—	—	—	—	—	Dec 28, 1928, expelled a dead calf (269 days), 8 guinea pigs inoculated with uterine material and 4 with colostrum failed to acquire abortion disease
CONTROL HEIFER 1425										
June 2, 1928	—	—	—	—	—	—	—	—	—	Apr 4, 1928, bred
July 18, 1928	—	—	—	—	—	—	—	—	—	
Aug 24, 1928	—	—	—	—	—	—	—	—	—	Aug 3, 1928, received 100 c c emulsion of fetal intestinal contents
Sept 19, 1928	+	+	+	+	+	—	—	—	—	Aug 9, 1928, received 100 c c emulsion of fetal intestinal contents
Oct 12, 1928	+	+	+	+	+	—	—	—	—	
Oct 27, 1928	+	+	+	+	+	—	—	—	—	
Nov 16, 1928	+	+	+	+	+	—	—	—	—	
Dec 6, 1928	+	+	+	+	+	—	—	—	—	
Dec 19, 1928	+	+	+	+	+	—	—	—	—	
Jan 8, 1929	+	+	+	+	+	—	—	—	—	
Jan 28, 1929	+	+	+	+	+	—	—	—	—	Dec 18, 1928, expelled a living calf (258 days); 6 guinea pigs inoculated with placental material and 6 with colostrum contracted abortion disease.
CONTROL HEIFER 1426										
June 2, 1928	—	—	—	—	—	—	—	—	—	Mar 24, 1928, bred
July 18, 1928	—	—	—	—	—	—	—	—	—	Apr 14, 1928, bred.
Aug. 24, 1928	P	—	—	—	—	—	—	—	—	Aug 3, 1928, received 100 c c emulsion of fetal intestinal contents
Sept 19, 1928	+	P	—	—	—	—	—	—	—	Aug 9, 1928, received 100 c c emulsion of fetal intestinal contents
Oct. 12, 1928	+	S	—	—	—	—	—	—	—	Nov 18, 1928, expelled a dead fetus (218 days); <i>Haem. abortus</i> isolated from fetus; 5 guinea pigs inoculated with colostrum contracted abortion disease.
Oct. 27, 1928	+	+	+	+	+	—	—	—	—	
Nov. 16, 1928	+	+	+	+	+	—	—	—	—	
Dec. 6, 1928	+	+	+	+	+	—	—	—	—	
Dec 19, 1928	+	+	+	+	+	—	—	—	—	
Jan. 8, 1929	+	+	+	+	+	—	—	—	—	
Jan. 28, 1929	+	+	+	+	+	—	—	—	—	

TABLE 5.—Records of individual experiment animals—Continued

CONTROL HEIFER 1453

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0 04	0.02	0 01	0.005	0 002	0 001	0 0005	0 0002	0.0001	
July 18, 1928	—	—	—	—	—	—	—	—	—	Breeding date not recorded
Aug. 24, 1928	—	—	—	—	—	—	—	—	—	Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents
Sept. 19, 1928	—	—	—	—	—	—	—	—	—	Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents
Oct. 12, 1928	—	—	—	—	—	—	—	—	—	Aug. 24, 1928, expelled a seem- ingly normal calf; 5 guinea pigs inoculated with placental material and 4 with colostr- um failed to contract abor- tion disease
Oct. 27, 1928	—	—	—	—	—	—	—	—	—	
Nov. 16, 1928	—	—	—	—	—	—	—	—	—	
Dec. 6, 1928	—	—	—	—	—	—	—	—	—	
Dec. 19, 1928	—	—	—	—	—	—	—	—	—	
Jan. 8, 1929	—	—	—	—	—	—	—	—	—	
Jan. 28, 1929	—	—	—	—	—	—	—	—	—	

DISCUSSION OF RESULTS

The three heifers receiving the vaccine of supposedly medium virulence (*Bacterium abortus* strain 19) gave evidence of not only resisting the localization of the infection in their bodies but of successfully withstanding the degree of *Bact. abortus* exposure to which they were subsequently subjected. One of the three heifers which received the vaccine that was regarded as most virulent (*Bact. abortus* strain 88) appeared to be rendered a more or less permanent carrier of the infection in the udder, and one of the five heifers receiving vaccine prepared from strains 8, 10, and 11 that had been under artificial cultivation longest failed to resist the invasion of the disease when later exposed. The limited data accumulated with reference to the proper virulence of *Bact. abortus* strains for calfhoo vaccination, therefore, suggested that strains of medium virulence might be superior either to those of prolonged artificial cultivation or to those of very recent isolation.

The breeding records of the animals used in the experiment failed to suggest that the use of the vaccine induced sterility, for only on rare occasions, as is indicated by the individual records, did either the principals or the controls fail to conceive on the first breeding.

While 2 of the 11 vaccinated animals eliminated *Bacterium abortus* in their colostrum either during their first parturition, or second, or both, Tables 3 and 4 show that 5 of the 10 controls from similar *Bact. abortus* exposure also became eliminators of the infection through this channel at times of calving or aborting. Since udder infection of but one principal could in fairness seemingly be charged to the vaccine injection, and since the most recently isolated strain of *Bact. abortus* was used in the preparation of the vaccine which this particular animal received, the method was not regarded as being seriously objectionable in this respect. The results of the experiment were, on the whole, encouraging.

CONCLUSIONS

The information which this calfhoo-immunization experiment yielded leads to the following conclusions:

It is possible, by the subcutaneous administration of abortion vaccine during calfhoo, to engender in bovines an immunity to

Bacterium abortus infection that is readily demonstrable during their first pregnancies.

The immunity afforded by early vaccination, possibly somewhat reinforced by *Bacterium abortus* ingestion exposures, seems to continue through second gestation.

Vaccine prepared with a *Bacterium abortus* strain of medium virulence may be preferable to those of very recent isolation or those of long-continued propagation on artificial media for calfhood-immunization purposes.

Vaccination during calfhood does not appear to produce effects that are manifested as sterility when the animals arrive at breeding age.

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THE CATALASE CONTENT OF THE COLORADO POTATO BEETLE DURING METAMORPHOSIS¹

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INTRODUCTION

In a previous paper, dealing with respiratory metabolism during the embryonic and metamorphic development of insects, the writer (5)³ pointed out several striking results in the gaseous exchange obtained during prepupal and pupal development. From an analysis of the data shown in Figures 6 to 10, inclusive, of that article, the inference was drawn that the curves represented the rate of metabolism during histolysis and pupal differentiation. That such an inference was justified is apparent from the marked diminution in the rate of gaseous exchange during the first few days of the prepupal condition, as illustrated by the descending phase of the curve and by the gradual increase in the rate of oxygen consumption and carbon-dioxide output during the last days of the prepupal period, as illustrated by the ascending phase of the curve.

In view of these investigations, it seemed to the writer a matter of considerable interest to determine whether other physiological phenomena of insects were markedly influenced during metamorphosis. An attempt was therefore made to observe the changes in the catalase content of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) during its histolysis and pupal differentiation.

REVIEW OF LITERATURE

There are no records in the literature which indicate determinations of catalase during the complete metamorphosis of an insect. Although Burge and Burge (4) investigated the catalase content of the potato beetle in different stages, no attempt was made by these writers to trace the course of activity of catalase during metamorphosis.

Other investigators have sought by experiment to determine the influence of certain hormones on the course of metamorphosis. Thus, Kopec (9), in one of a series of papers on the metamorphosis of insects, stated that the administration of thyroid extracts to caterpillars of *Lymantria dispar* did not cause any distinct changes in the duration of the larval or pupal period. From other experiments performed on caterpillars of the gipsy moth, Kopec (7, 8) concluded that probably, through the agency of internal secretion, the brain had the separate functions in hormonal action of causing histolysis of larval tissue and retarding the development of imaginal disks.

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² During the summer of 1927 the writer was assisted by I. R. Taylor and C. E. Herber, field assistants, in the experimental work described in this paper.

³ Reference is made by number (italic) to Literature Cited, p. 695.

Tiegs (12) holds that metamorphosis is brought about by a widespread death of tissue, due to an automatic starvation of larval cells. He argues that the cell contents, which increase as the cube of the radius of the growing cell, can not be nourished indefinitely through the cell membrane, the area of which increases only as the square of the radius. Bataillon (1), as the result of certain experiments on silkworms, decided that an accumulation of carbon dioxide in the tissues was an attendant factor in metamorphosis. This theory, however, has been disproved by the recent experiments of Singh-Pruthi (11), who placed larvae in a saturated atmosphere of carbon dioxide and found that this gas did not hasten metamorphosis but markedly retarded it.

MATERIAL AND METHODS

To observe the changes in catalase content during the histolysis, histogenesis, and pupal differentiation of the potato beetle, determinations of oxygen liberated from hydrogen peroxide by catalase were made with an apparatus essentially similar to the one described by Burge (3) in his research on the effect of work on catalase of muscle. Two inverted burettes, each of 50 c. c. capacity, were held in place by clamps and iron stands, the mouths of the burettes being set in a shallow pan of water and connected by means of glass tubing to a small flask in which oxygen was generated. By means of suitable screw clamps the flow of oxygen liberated by the action of catalase upon H_2O_2 in the generating flask could be diverted to one or the other of the two inverted burettes, and the volume of oxygen obtained by displacement of water in a given time was read on the scales of the burettes. The volumes of gas were reduced to standard conditions of temperature and pressure.

In view of the work of Morgulis, Beber, and Rabkin (10) and others, who have determined that destruction of catalase may occur when an excess of H_2O_2 is present, it seemed necessary to determine by preliminary experiment the minimum quantity of H_2O_2 to use in the investigations on the potato beetle. By preliminary trials with single larvae and pupae during 20-minute periods starting with various concentrations of superoxol from 0.5 to 5 per cent, and in other tests by also gradually increasing the quantity of superoxol solution from 10 to 30 cubic centimeters, it was found that at the end of 20 minutes no further oxygen was evolved when 30 cubic centimeters of a 3 per cent superoxol was used. This quantity was therefore adopted in all of the experiments with the potato beetle.

Mature, or nearly mature, larvae of the potato beetle, collected in the field, were brought into the laboratory and isolated in 1-ounce tin salve boxes containing damp sand in which the larvae might bury themselves when ready to pass through the prepupal period and transform to the pupa. The larvae in the salve boxes were kept at a temperature of 22° to 25° C. and examined twice daily, and a record was kept of the duration of the prepupal stage, the exact date of the formation of the pupa, and the development of the pupa.

In the experimental procedure for determination of catalase, a single larva was first weighed to the nearest milligram, ground in a mortar with a definite quantity of clean sand (washed and dried), and immediately transferred to the stoppered generating flask of the

apparatus already described; to the mixture was added 30 cubic centimeters of a 3 per cent solution of superoxol, previously adjusted to pH 7.0. The evolution of oxygen was observed as has been described.

Determinations of catalase were first made on mature larvae before they entered the soil for prepupation, and on material of each day of development thereafter throughout the prepupal and pupal stages. The experiments were conducted on three series of larvae collected during July and August. A series consisted of determinations of the oxygen liberated for each day of development, commencing with the mature larva, and extending through the prepupal and pupal periods. At least 10 determinations, conducted at room temperature, were made each day during the development of the insects in the series. In each determination the evolution of oxygen was limited to 20 minutes, the volume of gas collected being measured by successive readings for each of four consecutive 5-minute periods.

TABLE 1.—Oxygen liberated from hydrogen peroxide by the catalase of the Colorado potato beetle, at intervals of one day, during prepupal and pupal development

Stage of insect	Range of temperature during experiments	Insects	Average weight of insect	Average oxygen liberated per gram of insect in each of 4 consecutive 5-minute periods				Total oxygen liberated per gram in 20 minutes
				First period	Second period	Third period	Fourth period	
				Cu. cm.	Cu. cm.	Cu. cm.	Cu. cm.	
Mature larva.....	21-26	32	0.167	73.7	470.3	230.1	110.2	1,547.6
Prepupa								
First day.....	23-25	36	.147	586.1	316.1	223.1	143.7	1,290.0
Second day.....	23-25.6	37	.139	570.2	322.2	226.7	131.7	1,253.8
Third day.....	22-24.4	34	.150	600.4	427.9	313.9	192.7	1,534.9
Pupa (when formed)	21-23.6	31	.145	632.2	439.8	313.7	232.1	1,647.8
First day.....	22-24.6	30	.156	567.5	419.1	329.8	191.9	1,508.3
Second day.....	22-24.2	27	.148	571.5	412.1	331.6	205.5	1,520.7
Third day.....	23-25	40	.145	507.4	343.2	283.4	208.0	1,342.0
Fourth day.....	23-25.2	28	.141	439.7	304.7	268.3	212.3	1,225.0
Fifth day.....	23-24.5	26	.147	390.8	256.5	231.7	154.0	1,033.0
Sixth day.....	23-25.2	21	.148	293.5	193.6	148.7	117.3	753.1

EXPERIMENTAL DATA

To indicate the relative catalase content for each day of development of the insect, data from three complete series were averaged. Table 1 presents for each day of development the average volume, in cubic centimeters per gram of insect material used, of oxygen liberated in each of the 5-minute periods, and the total of these averages for the entire 20 minutes.

Striking differences in the proportion of catalase present at successive stages of development are suggested by the varying quantities of oxygen evolved per gram, as shown in each of the five columns of results. The quantities for the four 5-minute periods are presented graphically by the lower four curves in Figure 1, the totals for the 20-minute interval by the top curve. The most pronounced inhibition of activity of catalase was observed to occur on the first and second days of the prepupal period. As compared with the similar activity in the mature larva, the oxygen liberated in the 20-minute period on the first day of the prepupal stage was 16 per cent less, and

on the second day it was at its lowest recorded point of 19 per cent less. On the third and final day of the prepupal stage it was only 0.8 per cent less. On the following day, with the pupa formed, the evolution of oxygen in the 20-minute period reached its maximum, being 6 per cent greater than that for the mature larva, and exceeding it for the only time. Thereafter a steady decline was recorded until the lowest liberation of oxygen was obtained with the sixth day of the pupa

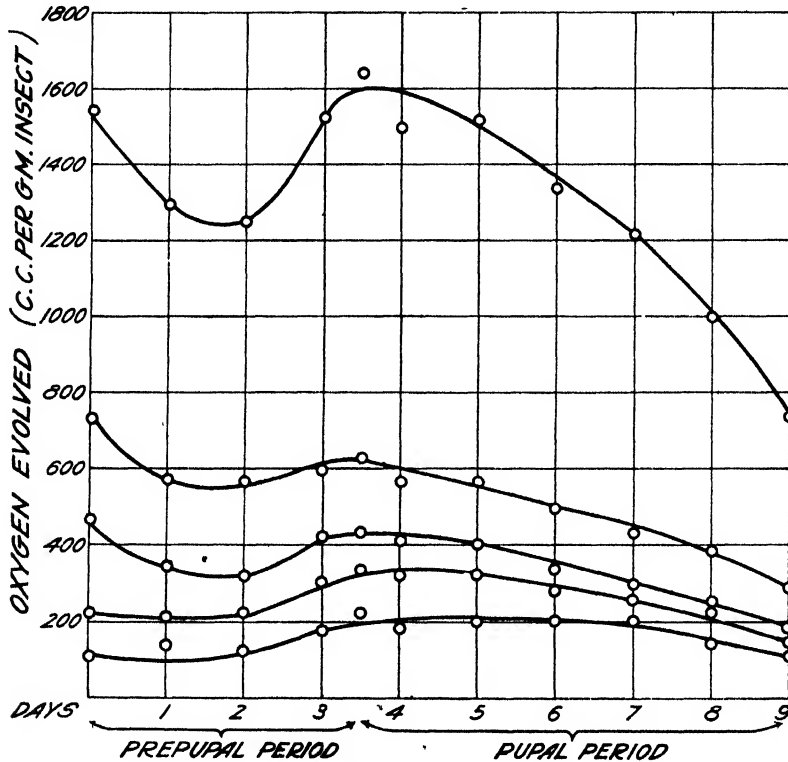


FIGURE 1—Average quantities of oxygen evolved from hydrogen peroxide by the action of catalase, in the Colorado potato beetle, for each day during the prepupal and pupal stages. The top curve shows the total oxygen produced during 20 minutes for each day of development. The four lower curves, in order, beginning at the top, indicate the average evolution of oxygen in four consecutive 5-minute periods, the sums of the ordinates for each day being the average total oxygen produced in the 20 minutes allowed for each individual determination

(formation of the adult), a reduction of 51 per cent from the rate for the mature larva.

Although the type of curve indicated for the oxygen released by catalase during histolysis very closely resembles the curve for CO_2 also evolved during histolysis, it is quite evident from the subsequent divergence of the former curve from that of the CO_2 during pupal development that they do not measure the same reaction. Burge and Burge (4) attributed the increase in the rate of oxidation in youth and its decrease in old age to the increase and decrease in catalase in the respective cases. Bodine (2) found that in the case of certain insects the content of catalase decreases with increasing age and with starvation, and that in hibernation there is a marked decrease in the

output of CO_2 with no corresponding change in catalase content, and Fink (6) found a correlation between the reduced activity of catalase and diminished respiratory metabolism in starving and hibernating insects, and that catalase activity of hibernating beetles is greatly reduced and their total catalase content is lower than that of old or young active beetles. It is agreed among physiologists that increased excretion of CO_2 may be considered a satisfactory index of development, since that excretion is generally accepted as an index of metabolizing tissue. The excretion of CO_2 is also a far better index of growth than increase in weight, since the latter may also be due to an increased content of inert or nonliving substances. Catalase content, however, is not an index of growth or development, since the curve produced by its action on hydrogen peroxide does not parallel that of CO_2 during the entire development, although it approximately does so during histolysis. The content of catalase seems to decrease during the pupal development, whereas the excretion of CO_2 increases.

The extensive disruption of tissue during histolysis may account partly for the decreased content of catalase manifested during the first few days of the prepupal period. If, as pointed out by other investigators, the function of the catalase is to prevent the formation of hydrogen peroxide in the living tissue, the low catalase content usually measured during histolysis might indicate that during this period an exceptionally large supply of catalase was required by unhistolyzed tissue to neutralize the excess peroxide formed during the process of histolysis, and that the catalase found at this time was simply that remaining after the dissociation of the peroxide in the living tissue.

SUMMARY

The variation in the activity of catalase during the metamorphosis of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), beginning with the mature larva, was ascertained by making daily determinations of the quantity of oxygen per gram of tissue evolved from hydrogen peroxide by the action of catalase during prepupal and pupal development.

Analysis of the resulting data indicated a reduction in catalase of 16 per cent on the first day of histolysis and 19 per cent on the second, followed on the third day of histolysis by an increase which, when the pupa was formed, reached the maximum of 6 per cent above that of the mature larva. The decline in content of catalase during pupal development was continuous thereafter until a reduction of 51 per cent below that of the mature larva was reached with the formation of the adult.

The curves here obtained for catalase content and those previously reported by the writer for oxygen consumption and excretion of carbon dioxide (5) were similar for the prepupal period of histolysis. During pupal development, however, the catalase curve declined rapidly, whereas the curves for oxygen and carbon dioxide followed the usual growth curve.

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DEVELOPMENT OF AXILLARY BUDS ON FRUITING BRANCHES OF PIMA AND UPLAND COTTON¹

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INTRODUCTION

The cotton plant produces two distinct types of branches, which occupy different positions and have different structures and functions. These are the upright "limbs" or vegetative branches, which do not produce flowers or bolls, and the fruiting branches, which assume horizontal positions and on which are developed the floral buds. Cook² has shown that in addition to the bud that serves to continue the growth of the shoot, each node of the main stalk produces two other buds, one in the axil of the leaf and another to the right or left of the axil, called an extra-axillary bud. The axillary buds usually remain dormant, but may be developed into vegetative branches when conditions for luxuriant growth are favorable. The fruiting branches arise from the extra-axillary buds on the main stalk and on vegetative branches.

The fruiting branches also produce two buds at each node, corresponding to the two types on the main stem. These are a flower bud, which appears between the bases of the stipules, and an axillary bud, which is formed in the axil of the leaf. It has been observed that the axillary buds on the fruiting branches of Egyptian cotton differ somewhat in morphology and behavior from those on the upland type. These distinctions and their possible relationship to cultural problems and studies in bud shedding are presented in this paper.

COMPARISON OF AXILLARY BUDS ON EGYPTIAN AND UPLAND COTTONS

Under ordinary conditions of growth a greater part of the axillary buds on the fruiting branches of upland cotton remain dormant, but in cases of exceptional luxuriance, or when the terminal growth of the branches or main stem is injured, a considerable number of them may undergo further development and produce short vegetative branches. (Fig. 1.)³

In Egyptian cotton the axillary buds on the fruiting branches seldom if ever remain dormant for any great length of time. They emerge from their positions in the axils of the leaves as minute triangular buds or "squares," about two or three days after the extra-axillary bud on the same node, and at first in all respects appear

¹ Received for publication July 17, 1930; issued November, 1930.

² COOK, O. F. MORPHOLOGY OF COTTON BRANCHES. U. S. Dept. Agr., Bur. Plant Indus. Circ. 109: 11-16, illus. 1913.

³ The photographs illustrating this paper were made by H. F. Loomis.

like normal fruit buds. They are unlike the axillary buds of upland cotton in that they are usually unattended by a leaflet with stipules. (Fig. 2.) Under ordinary conditions their growth continues for

only a few days, and then they drop off or begin to shrivel and finally dry up into minute dark-colored bodies, many of which remain attached to the fruiting branches.

If the plants are especially productive or if the terminal growth of branch or main stem is aborted or injured, a number of the axillary buds may continue development. When this occurs there usually is produced a single boll on a long stem (fig. 3), but occasionally two or more bolls may develop. Some of the boll stems have the appearance of simple pedicels, but many of them show some type of joint and occasionally small bract-like leaves or stipules, which indicate that the "pedicels" comprise shortened branches. Cook⁴ points out that in reality three independent elements are represented in such stems — an axillary branch, a fertile branch from the axillary, and the pedicel of the boll, all fused into a simple stem.

This type of fusion is



FIGURE 1 —A fruiting branch (in two sections) of *Acala* (upland) cotton, showing development of axillary buds at every node. This development occurred late in the season, long after the shedding of extra-axillary bolls on the first four nodes, whose positions are shown by the scars. At node 5 the largest bud shown is the extra-axillary, and the small bud to the left is the axillary. At node 6 only the extra-axillary bud is visible.

carried much farther in Egyptian cotton than in upland cotton. In upland cotton the boll stems invariably have a well-defined joint to indicate that a branch is involved. (Fig. 4.)

⁴ COOK, O. F. DIMORPHIC BRANCHES IN TROPICAL CROP PLANTS: COTTON, COFFEE, CACAO, THE CENTRAL AMERICAN RUBBER TREE, AND THE BANANA. U. S. Dept. Agr., Bur. Plant Indus. Bul. 198, 64 pp., illus. 1911.

FACTORS THAT AFFECT DEVELOPMENT OF AXILLARY BUDS

The difference in the behavior of the axillary buds in the two types of cotton is of importance in relation to some of the production problems that are peculiar to certain of the irrigated valleys of the Southwest. In these regions the growing seasons are long, but the cotton plants are frequently forced to undergo periods of severe

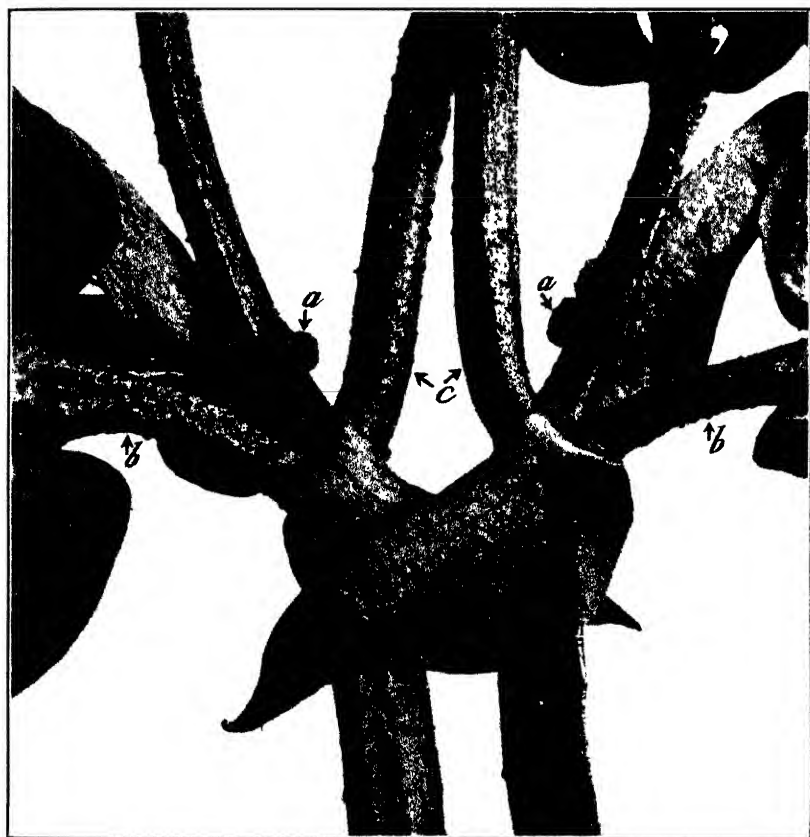


FIGURE 2—Relative positions of extra-axillary and axillary fruit buds at each node on the fruiting branches of Pima Egyptian cotton plants. The small axillary buds or squares can be seen, at the base of the leaf petioles, as they appear when three or four days old, and are about ready to drop off or shrivel up. It will be noted that these buds usually do not develop the subtending leaf and stipules which characterize this type of bud in upland cotton *a*, Axillary bud, *b*, petiole of extra-axillary bud, *c*, petiole of leaf

stress, resulting from improper water relations when high temperatures prevail. Since the axillary buds on the fruiting branches of Egyptian cotton do not remain dormant and ordinarily survive for only a few days, it is apparent that any stimulus that would influence the retention and further development of these buds must be exerted before or during the few days when they are in full vigor of growth.

With upland cotton, on the other hand, the establishment of the proper conditions can apparently stimulate the growth of the dormant axillary buds at any time during the growing season, especially if the plants have previously thrown off a large part of their extra-axillary

fruit. In some of the hot interior valleys of the irrigated region the upland varieties are prone to undergo excessive shedding of buds and young bolls during periods of high temperature, and in some seasons many of the plants may shed all of the bolls and buds that

are in the early stages of development during such periods. It has been observed that after climatic conditions have moderated near the close of an especially hot and unfavorable summer many of the upland plants that have undergone excessive shedding may begin to develop at almost every node, these short axillary branches bearing one or more floral buds. (Fig. 5.) Some even emerge from the first nodes near the main axis, where in rare cases a mature boll may be found on the same node.

Not infrequently it may be observed that two cycles of flowering are in progress on the same fruiting branches, some of the later nodes bearing extra-axillary flowers while the older inner nodes may carry flowers on short axillary branches. (Fig. 6.) While under Arizona conditions a large number of these tardy floral buds may reach the flowering stage, it is only seldom that they reach maturity, on account of injury by frosts, and conse-



FIGURE 3.—Axillary and extra axillary bolls of Pima cotton, showing difference in type of boll stems. The axillary bolls usually have long stems and often show some semblance of a joint

quently they fail to play any important part in the final yield. However, where the seasons are longer, as in the valleys of southern California, it has been observed that in some seasons late-matured bolls from short axillary branches contribute materially to the yield.

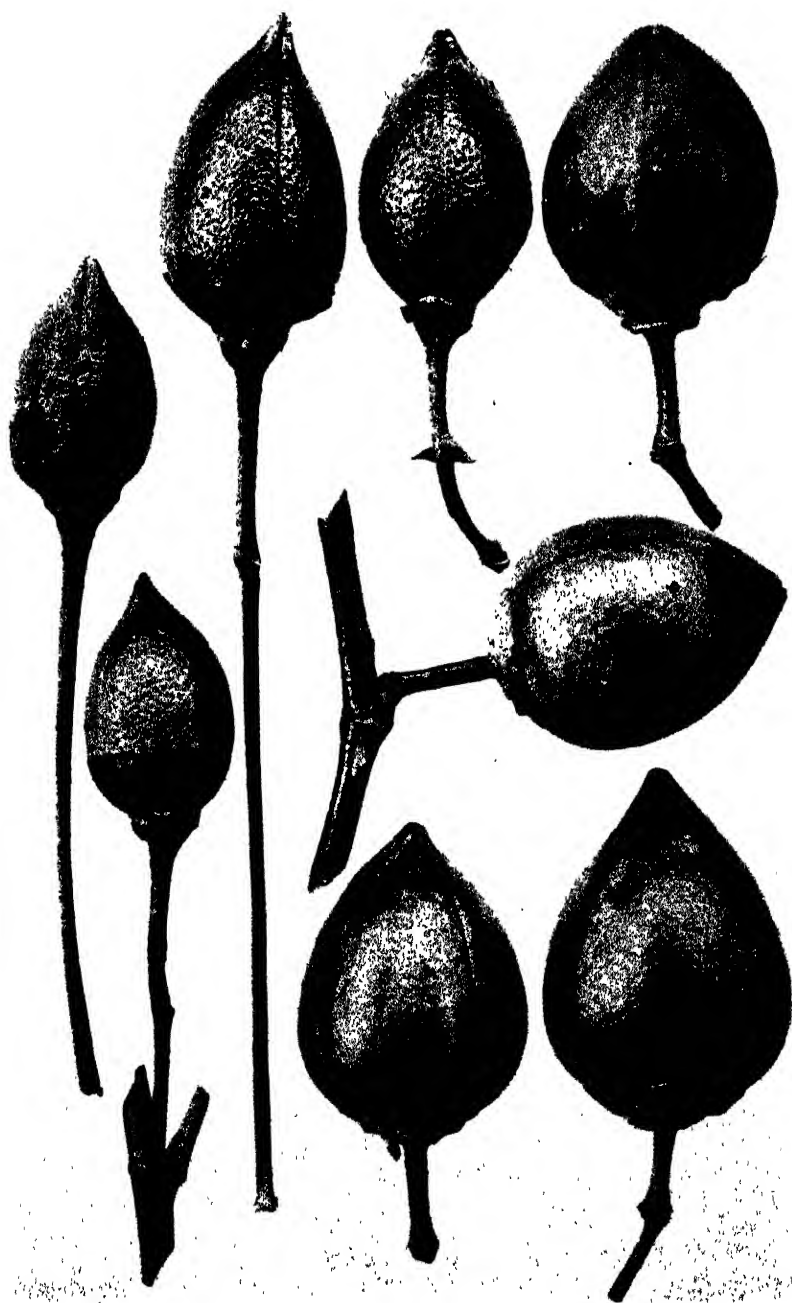


FIGURE 4.—Axillary bolls of Pima and Acala cotton, illustrating different types of pedicels. The four Pima bolls shown at the left and top center are smaller and have longer stems and are more deeply pitted than the four Acala bolls

EFFECT OF REMOVAL OF EXTRA-AXILLARY BUDS ON DEVELOPMENT OF AXILLARY FRUIT

Experiments were begun in 1926 to determine to what extent the retention and development of axillary buds could be influenced by



FIGURE 5 A fruiting branch of Acala cotton (in two sections), showing an axillary boll developing at each node late in the season. The extra-axillary fruit had shed naturally from the first four nodes, and the scars are clearly shown. The flower at the fifth node is from an extra-axillary bud

the removal of the fruit buds in the extra-axillary positions. In this season the defruiting process was not started until June 17, after the plants had already developed several fruiting branches, some of which had borne buds that had almost reached the flowering stage.

Twenty-seven normal Pima plants were selected from a group of well-grown plants in a single row about 200 feet long, and all the fruit buds were removed from 17 of them on that date. Ten other plants of about equal size, interspersed among these 17, were selected as controls and tagged for observation as to the development of axillary fruit under normal conditions. In an adjacent row planted to the Acala variety 10 plants were selected on the same day and 5 of them were defruited, the other 5 being designated as controls. From June 17 until September 1 the extra-axillary fruit buds on the 17 defruited Pima plants and the 5 defruited Acala plants were pinched out every

two or three days and a tag was attached to the internode to show the date of removal. On October 11, diagrams were made of all of the plants to show the location of the axillary fruit and the number

of vacant nodes. The data obtained from these diagrams are shown in Tables 1 and 2.

In 1928 a more careful study of axillary fruit production was conducted on 10 Pima and 9 Acala plants from which the extra-axillary



FIGURE 6 Fruiting branches of Acala cotton, showing axillary buds ready to flower, on nodes behind flowers and bolls that have been developed in the extra-axillary positions. Thus it sometimes happens on upland cotton that a second wave of flowering may be in progress after that which normally occurs

buds were removed daily and on 10 control plants of each variety which were allowed to grow normally. The defruiting process was started on June 1 and discontinued on September 4. The influence of this defruiting on the plants is shown by the data in Tables 3 and 4.

TABLE 1.—Growth of main stem, number of nodes and axillary buds developed, axillary buds shed and percentage retained on Pima and Acala cotton plants on which the extra-axillary buds were removed every two or three days during the season, at the United States Field Station, Sacaton, Ariz., in 1926

Variety and plant No.	Height of plant	Nodes on fruiting branches	Axillary buds recorded on fruiting branches June 17 to Sept. 1	Axillary buds recorded as shed before flowering	Axillary buds retained to Oct. 11	Axillary bolls matured on main stem	Extra-axillary buds removed during season
	Inches	Number	Number	Number	Per cent	Number	Number
Pima:							
1.....	72	109	64	16	75.0	12	116
2.....	70	120	51	25	51.0	9	99
3.....	73	118	44	25	43.2	4	95
4.....	68	84	31	12	61.3	4	71
5.....	73	138	52	21	59.6	4	131
6.....	78	146	55	27	50.8	10	118
7.....	69	102	43	22	48.8	5	91
8.....	66	68	28	10	64.3	7	58
9.....	73	99	39	15	61.5	5	88
10.....	80	126	44	8	81.8	6	101
11.....	72	94	33	17	48.5	2	74
12.....	81	264	83	20	61.2	17	176
13.....	84	197	124	55	55.7	15	147
14.....	74	187	106	46	56.6	15	138
15.....	79	133	77	40	48.1	11	109
16.....	78	190	85	43	49.4	10	152
17.....	80	163	87	38	56.3	10	132
Mean.....	74.7±0.85	141.1±8.25	61.5±4.60	25.9±2.43	57.24±1.68	8.6±0.74	111.52±5.26
Acala:							
1.....	75	265	146	66	54.8	5	185
2.....	65	267	163	78	52.2	23	197
3.....	61	265	141	84	40.4	8	171
4.....	69	216	108	66	38.9	3	166
5.....	68	225	127	84	33.9	4	170
Mean.....	67.6±1.65	247.6±8.01	137.0±6.64	75.6±2.92	44.04±2.80	8.6±2.65	177.8±4.14

TABLE 2.—Growth of main stem and number of nodes and axillary and extra-axillary bolls produced on normally developed Pima and Acala cotton plants at the United States Field Station, Sacaton, Ariz., in 1926 *

Variety and plant No	Height of plant	Nodes on fruiting branches	Axillary bolls matured on fruiting branches	Axillary bolls matured on main stem	Extra-axillary bolls matured
	Inches	Number	Number	Number	Number
Pima:					
1.....	66	79	0	0	47
2.....	61	108	4	0	73
3.....	65	118	1	0	85
4.....	60	49	0	1	40
5.....	66	64	0	0	44
6.....	61	70	0	0	52
7.....	51	50	0	0	33
8.....	65	65	1	0	39
9.....	58	43	0	0	27
10.....	58	56	1	1	38
Mean.....	61.4±1.03	70.2±5.50	0.7±0.213	0.2±0.09	47.8±3.96
Acala:					
1.....	56	102	0	0	21
2.....	59	120	0	0	35
3.....	65	114	0	0	25
4.....	59	99	0	0	28
5.....	64	130	0	0	36
Mean.....	60.6±1.21	113.0±4.11	0	0	29.0±2.07

* The total number of axillary buds which developed and shed on the normally developed plants was not recorded in 1926.

TABLE 3.—Number of nodes, axillary buds developed, axillary buds shed, and percentage retained on Pima and Acala cotton plants on which the extra-axillary buds were removed daily during the season, at the United States Field Station, Sacaton, Ariz., in 1928 ^a

Variety and plant No.	Nodes on fruiting branches	Axillary buds recorded on fruiting branches June 1 to Sept. 4	Axillary buds recorded as shed before flowering	Axillary buds retained to flowering	Extra-axillary buds removed during season
	Number	Number	Number	Per cent	Number
Pima					
1	214	150	120	20.0	175
2	184	125	99	20.8	160
3	173	127	106	16.5	158
4	176	120	93	22.5	155
5	162	107	96	10.3	144
6	155	90	81	10.0	136
7	176	118	81	51.4	158
8	150	87	74	14.9	133
9	189	117	93	20.5	172
10	199	136	115	15.4	169
Mean	177.8±4.32	117.7±1.23	95.8±3.27	18.23±1.39	156.0±2.93
Acala					
1	143	90	63	30.0	114
2	130	54	31	42.6	113
3	255	161	97	39.8	205
4	116	51	39	27.8	92
5	99	47	31	34.0	74
6	137	51	34	33.3	92
7	173	64	28	56.3	115
8	119	33	4	87.9	78
9	127	39	17	56.4	87
Mean	144.3±10.73	65.9±9.09	38.2±6.20	45.34±4.22	107.8±9.18

^a The height of plants and the number of axillary bolls matured on main stem were not recorded in 1928.

TABLE 4.—Number of nodes, axillary buds developed, axillary buds shed, percentage retained to flowering, and number of extra-axillary buds retained, on normally developed Pima and Acala cotton plants at the United States Field Station, Sacaton, Ariz., in 1928 ^a

Variety and plant No.	Nodes on fruiting branches	Axillary buds recorded on fruiting branches June 1 to Sept. 4	Axillary buds recorded as shed before flowering	Axillary buds retained to flowering	Extra-axillary buds retained to flowering
	Number	Number	Number	Per cent	Number
Pima					
1	79	47	44	6.4	20
2	80	53	51	3.8	36
3	68	42	42	0	29
4	78	40	40	0	24
5	69	38	37	2.6	28
6	71	37	36	2.7	24
7	64	34	32	5.9	24
8	53	30	29	3.3	20
9	51	25	25	0	25
10	63	36	35	2.8	25
Mean	67.6±2.23	38.2±1.76	37.1±1.66	2.75±.503	25.5±1.02
Acala					
1	98	2	2	0	50
2	78	5	5	0	38
3	93	0	0	0	29
4	109	22	22	0	31
5	90	4	4	0	36
6	81	1	1	0	34
7	87	5	4	20.0	37
8	104	2	2	0	54
9	98	1	1	0	47
10	96	6	6	0	41
Mean	93.4±2.13	4.8±1.40	4.7±1.39	2.0±1.39	39.7±1.81

^a The height of plants and the number of axillary bolls shed after flowering were not recorded in 1928.

It will be observed from these data that the removal of the fruit buds in the extra-axillary positions caused the plants to grow taller than normally and to produce a much greater number of internodes. The fruiting branches of the defruited plants were much longer than

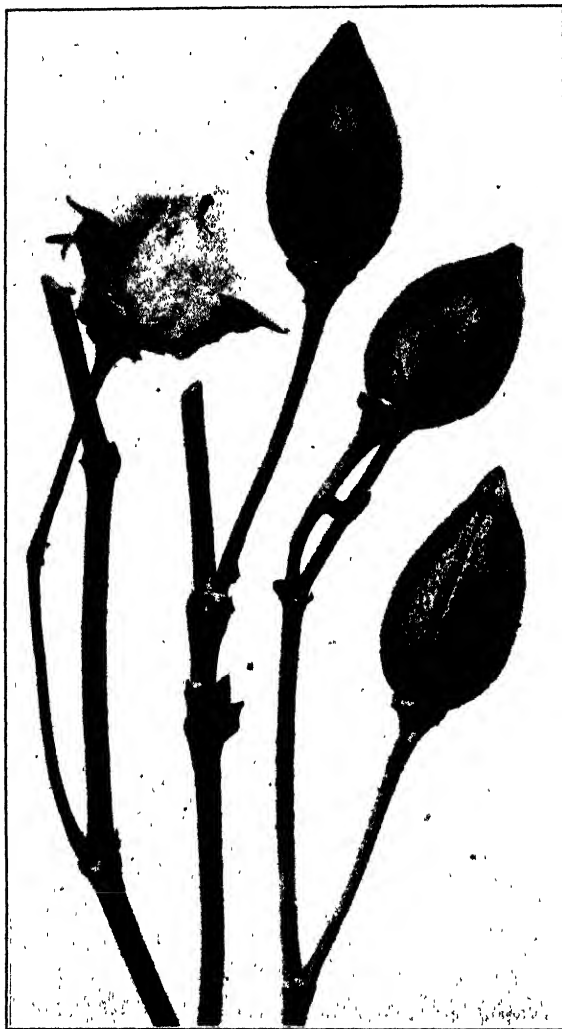


FIGURE 7—Axillary bolls produced on a single fruiting branch of a Pima cotton plant on which no extra-axillary fruit was allowed to develop

those of the normal plants and a greater number of fruiting branches was developed. In 1926 the mean number of internodes produced on the defruited Pima plants was 141 ± 8.3 , while the mean number on the normal Pima plants was 70 ± 5.5 . Since the observations were not continued throughout the season, the number of axillary buds recorded in the tables does not represent the total number that developed, as many buds had appeared and shed before the observations were begun, and a larger number appeared after September 1. In 1926 an average of 111 ± 5.3 extra-axillary buds were removed from the Pima plants and 178 ± 4.1 from the Acala plants, and in 1928 an average of 156 ± 2.9 buds per plant were removed from the Pima plants and 108 ± 9.2 from the Acala plants. A large proportion of axillary buds were developed to maturity on some of the defruited

plants; in some cases three or four bolls were matured on a single branch. (Fig. 7.) In 1926 the mean percentage retained until the end of the season was 57.2 ± 1.7 for Pima and 44.0 ± 2.9 for Acala. The effects of the defruiting on the retention of the axillary buds did not become apparent on the Pima until the process had been in progress for some time. Very few axillary bolls were developed on the



FIGURE 8.—Pima cotton plant showing bolls produced from axillary buds. The extra-axillary buds were removed every two or three days during the season.

lower branches of the Pima plants, and at the end of the season most of the axillary bolls retained were found on the outer nodes of the middle branches and on the late-developed branches at the top.

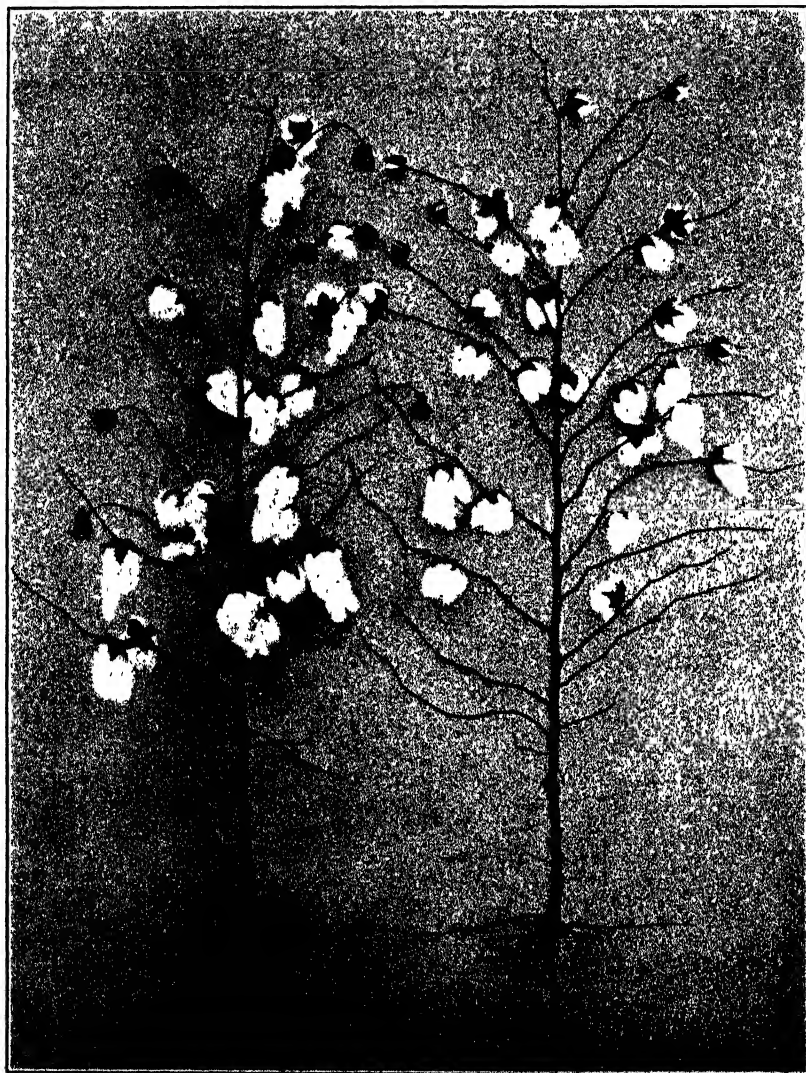


FIGURE 9.—Two Acala cotton plants that have produced bolls from axillary buds only. The extra-axillary buds were removed daily soon after they appeared. Note that many of these bolls are developed on nodes close to the main axis, while most of those on Pima cotton (fig. 8) developed on outer nodes and near the top of the plant. This is due to the fact that axillary buds on upland cotton usually remain dormant and may be stimulated to growth at any time, while on Pima cotton the axillary buds are shed or dry up on the plants a few days after appearance unless some stimulus occurs to force them into further development.

(Fig. 8.) With the Acala variety, however, many of the axillary buds on the inner nodes of the lower branches became active after defruiting had been carried on for a time, and at the end of the season a considerable number of matured axillary bolls were found on the

lower and middle branches, even on the nodes near the main axis. (Fig. 9.)

It will be observed that very few axillary buds developed to maturity on the normal plants of Pima or Acala in either 1926 or 1928. However, plants grown under the most favorable conditions often develop to maturity a considerable number of axillary bolls in addition to the extra-axillary crop. In 1927 a count showed that the mean number of axillary bolls matured on five large, wide-spaced Pima plants was 6.4, in addition to a mean of 68.2 extra-axillary bolls.

In Arizona certain varieties show a greater tendency than others to produce axillary fruit. Plants of the sea-island type, which in this region usually do not produce any bolls until late in the season, often produce large numbers of axillary bolls on the upper fruiting branches and on the outer nodes of older branches. In 1927, 10 representative sea-island plants set an average of 9.3 axillary bolls per plant. The native southwestern varieties, Hopi and Sacaton Aboriginal, also show a tendency to produce an unusual amount of axillary fruit. Diagrams made of 10 Sacaton Aboriginal plants in 1927 showed an average of 10.2 axillary bolls per plant.

AXILLARY BUD DEVELOPMENT ON PLANTS AFFECTED WITH CRAZY-TOP DISORDER

In recent years there has appeared in many cotton fields of the Salt River Valley a peculiar disorder known as crazy top, which causes sterility and results in many abnormalities of the affected plants. In studying the effects of this disorder on the plants it has been observed that upon the arrival of cooler and more favorable weather conditions in the fall some of the more luxuriant upland plants that have been almost sterile from crazy top will begin to develop axillary branches and bear floral buds at many of the nodes from which extra-axillary bolls have been shed. Pima plants that have been rendered practically sterile by the disorder, although having no reserve axillary buds on the lower internodes of the branches as do the upland plants, are able to produce "supernumerary" bolls late in the season on new joints, by retaining a large proportion of the axillary buds. Frequently large clusters of bolls are observed in the tops of Pima plants affected with crazy top as a result of this tendency toward recovery. (Fig. 10.) As in the case of sterility from normal stress effects, the development of this fruit from axillary branches occurs late in the season, and it is not often that enough of it matures to be a factor in production. However, it is of interest from the standpoint of physiology and from its indication that there is a tendency toward recovery from the disease.

SHEDDING OF AXILLARY BUDS ON EGYPTIAN COTTON

It would appear that some investigators have failed to recognize that there is a difference in the morphology of the Egyptian and upland cotton plants with respect to the axillary buds. In studying the relative proportions of buds, flowers, and bolls from 1,300 Egyptian plants at Giza, in Egypt, Balls⁵ collected weekly all the mate-

⁵BALLS, W. L. THE COTTON PLANT IN EGYPT. 202 p., illus. London. 1912.

rial beneath the plants and presented an analysis of the results in the form of shedding curves.

Bailey and Trought,⁶ working with the Ashmouni, Assili, and Sakellaridis varieties, found "that the great majority of buds fall off at a very early stage when the bud is only about 2 mm. or less in width measured across the epicalyx." In an experiment carried out in 1923 they reported that the number of buds and bolls which were shed by 40 Sakellaridis plants during the period June 26 to September 26 was as follows:

Small buds (not more than 5 mm. in diameter).....	787
Larger buds	275
Bolls (all ages).....	92

From these data they concluded that "about 60 per cent of the 'possible crop' was lost in this case through bud shedding, while

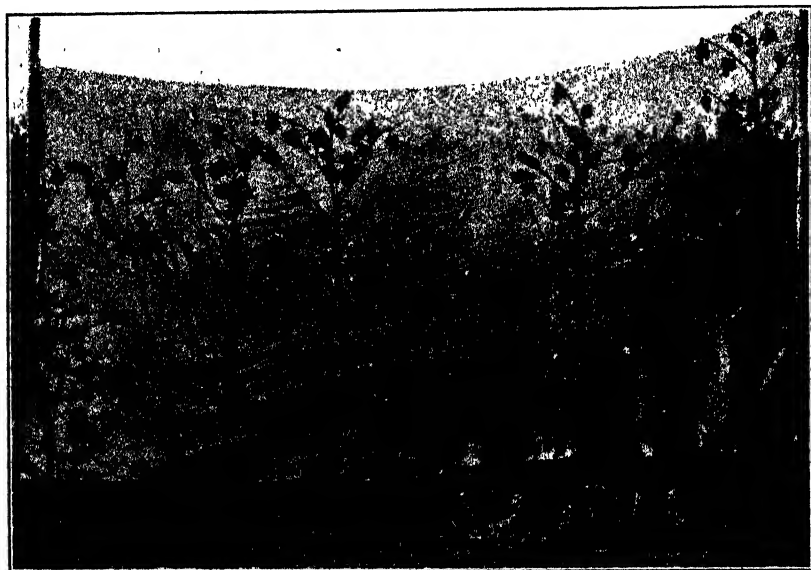


FIGURE 10 — Pima cotton plants as they commonly appear late in the season when affected by the crazy-top disorder. It will be noted that most of the bolls that are set are on the terminal nodes. A part of the crazy-clustered appearance of the bolls is due to the retention of the axillary fruits.

boll shedding accounted for about 6 per cent of the total." In this experiment, as in those conducted in 1921 and 1922, these investigators used the "catch-net" system of collecting all of the buds that dropped off each day and classified them into ages according to size.

The experiments conducted by the writer indicate that in a study of bud shedding the method of collecting the fallen buds from the ground or in catch nets is open to serious error. Should this method be followed with Pima Egyptian cotton, the number of squares falling into the class measuring less than 5 mm. across the epicalyx would be far too high if considered as an actual loss of "possible crop," since it is never possible to obtain two bolls at each node throughout the plant, no matter how favorable the conditions.

⁶ BAILEY, M. A., and TROUGHT, T. GROWTH, BUD-SHEDDING, AND FLOWER PRODUCTION IN EGYPTIAN COTTON. Egypt Min. Agr., Tech. and Sci. Bul. 65, 8 pp., illus. 1927.

Obviously, many of the rudimentary axillary buds, which emerge with as great regularity as the "normal" buds, but which only rarely develop into bolls, would be included in the count, since under ordinary conditions they drop off when at a size of from 1 to 5 mm. across the epicalyx, though many shrivel up and remain attached to the plants for long periods.

The great similarity of these axillary buds and the extra-axillary buds would make it impracticable to distinguish between the two kinds if all were collected together. (Figs. 11 and 12.) It will be seen from Table 4 that the mean number of axillary squares developed on the normal Pima plants between June 1 and September 4, 1928, was 38 ± 1.8 . Practically all of these were shed, and had they been counted with the shed extra-axillary squares the total number would have been nearly twice as great as the mean number of vacant extra-axillary nodes on the plants.

SUMMARY

The axillary buds and branches on the fertile branches of Egyptian cotton differ in morphology and behavior from those on upland cotton.

In the Pima variety of Egyptian cotton rudimentary axillary branches begin development with the advent of minute triangular buds in the axil of each leaf, but ordinarily this development is cut short by the shedding or drying up of the buds, which maintain active growth for only a few days.

In upland varieties the axillary buds usually remain dormant, but they can be stimulated to growth at any time later in the season.

In Arizona, axillary buds on upland plants frequently begin development late in the season, especially on luxuriant plants that have shed excessively during the summer, and a second cycle of flowering may occur on the older fruiting branches.

The axillary buds and bolls on both types of cotton are usually developed too late to contribute materially to the yield under Arizona conditions.

Removal of the extra-axillary buds artificially by pinching off the new buds at frequent intervals resulted in more axillary buds being retained by the Pima plants and many of them continued development to maturity.

The removal of the extra-axillary buds on upland plants stimulated many of the axillary buds into development, and a considerable number of them were retained by the plants until maturity.

On the defruited Pima plants the axillary fruits that matured were located only on the internodes developed late in the season, while on the defruited upland plants a greater part of them matured on the internodes developed earlier in the season.

The artificial removal of the extra-axillary buds caused the plants to grow much taller and to produce longer and more numerous fruiting branches than normally.

Both Pima and upland plants that have been rendered almost sterile during the summer from effects of the crazy-top disorder may develop a large number of axillary fruits late in the season, those on

the upland plants being produced on both early and late developed internodes, while those of Pima form only on the late growth.

The large numbers of very small buds that are shed naturally from the axillary positions on Egyptian cotton make it impracticable to use the method of collecting the shed squares or young bolls as a basis for estimating the "possible crop" or for assigning a proper ratio of shed buds and bolls, as has been attempted by some investigators.

LINUM NEOMEXICANUM (YELLOW PINE FLAX) AND ONE OF ITS POISONOUS CONSTITUENTS¹

By W. W. EGGLESTON, Assistant Botanist, O. F. BLACK, Senior Biochemist, and J. W. KELLY, Junior Biochemist, Office of Drug and Related Plants, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The senior writer, while examining plants on a range where cattle had been poisoned about Willow Spring tank, on the western slope of Sitgreaves Mountain, Tusayan National Forest, Ariz., noticed small patches of yellow pine flax (*Linum neomexicanum*). A few days later, in the Tonto National Forest, this same species was found in abundance along the old wagon road from Pine, Ariz., to Flagstaff, near the top of Mogollon Rim. This wagon road was in use for many years as a stock trail and for freighting purposes, but is now paralleled by an automobile road. The place where the flax grew in greatest abundance was the site of an old camp ground, and much stock had been poisoned in that vicinity. Although there was no direct evidence to connect *L. neomexicanum* with these fatalities, samples were collected and sent to the laboratory, where they were examined, with the result that a poison of glucosidal nature was isolated. The object of the present paper is to give a botanical description of the plant and some facts concerning the poison derived from it.

In the fall of 1880 E. L. Greene discovered this interesting species while living at a little mining camp of Pinos Altos in the Pinos Altos Mountains, Grant County, N. Mex., and described it in the Botanical Gazette of March, 1881.²

The plant is now known in New Mexico, north to the middle and west forks of Gila River, east to the Black Range, and south in the Big Burro and San Luis Mountains to Guayanopa Canyon in the Sierra Madre de Chihuahua. In the southeastern Arizona it is known in the Chiricahua Mountains, the Huachuca, the Rincon, the Santa Rita, the Pinal and the Mule Mountains, and extends northwestward along Mogollon Rim to Sitgreaves and Cedar Mountains.

Two other wild flaxes are found in the grazing country, and both of them are suspected of poisoning sheep. One, *Linum lewisii*, a blue-flowered flax, occurs on the higher mountain ranges; the other, *L. rigidum*, a yellow-flowered species, occurs in the Pecos Valley in Texas.

BOTANICAL DESCRIPTION

Linum neomexicanum Greene. Yellow pine flax. (Fig. 1.) Annual, or winter annual herb, flowering in August, erect, glabrous, slightly glaucous, stems simple, branched from near the base, slender, angled, 1 to 2 feet tall; lower leaves opposite below, becoming alternate above, erect or ascending, sessile, glabrous, about one-half inch long, lanceolate, oblong, entire, acute, crowded near the base, becoming narrower and farther apart above, bracts lanceolate to linear; pedicels one-fourth to one-half inch long; sepals 5, persistent, lanceolate to ovate, acuminate.

¹ Received for publication July 15, 1930; issued November, 1930.

² GREENE, E. L. NEW PLANTS OF NEW MEXICO AND ARIZONA. Bot. Gaz. 6. 183-185. 1881.



FIGURE 1.—*Linum neomexicanum* (yellow pine flax)

nate, glandular, serrate or entire, about one-fourth inch long, shorter than the carpels; petals 5, yellow, one-fourth inch long; stamens 5, longer than the sepals, united at the base; filaments linear-lanceolate; capsules ovoid, incompletely 10-celled, one-fourth inch wide, unribbed; seeds flattened, oblong in outline, light brown, shining.

Distribution.—New Mexico, Arizona, and Chihuahua (Mexico).

Altitude.—5,000 to 8,000 feet.

TOXICITY

No member of the family Linaceae has hitherto been proved to contain material that is actively poisonous, although it is well known that some of them possess the cyanogenetic glucoside linamarin. Glucosides of this type, however, are found in other plants commonly used for forage, as, for instance, sorghum, and in feedstuffs, as flax-seed cake, which is frequently used as a concentrate for fattening cattle, although it usually contains small amounts of hydrocyanic acid. *Linum catharticum*, so far as the writers have been able to discover, is the only species of flax known to possess physiologically active properties, and even in this case it is a matter of dispute whether the substance found, linin, has the purgative effects that were earlier attributed to it.³

EXPERIMENTAL WORK

The air-dried plant was ground to moderate fineness and 50 gm. was tested for the presence of hydrocyanic acid by distillation with acid, and the distillate was treated for Prussian blue. The results, however, were negative. The same procedure was repeated after the ground material had been allowed to macerate for an hour in the presence of emulsin. This also yielded a negative result. These tests, of course, do not show conclusively that the fresh plant does not contain hydrocyanic acid, but the probabilities are that it does not.

As a preliminary test for toxicity, an aqueous extract was prepared by steeping 60 gm. of the plant in successive portions of hot water until the material was thoroughly exhausted of water-soluble substances. The water extract was evaporated on the steam bath to small bulk, filtered, and made up to exactly 100 c. c. One-half of this, representing 30 gm. of the plant, was fed to a rabbit per os by means of a stomach tube. The animal seemed little affected, although it was inert and dejected during the day, but that night it died. Next, a white mouse was given subcutaneously one-fourth c. c. of the same solution, equivalent to 0.15 gm. of the plant, and manifested the same behavior, dying within 18 hours. The results indicated that the plant contains a poisonous substance or substances.

To gain a clearer idea of the nature of this poisonous material, 180 gm. of the ground plant was extracted in a Soxhlet apparatus, first with ether, then with alcohol, and finally with water. All three of these extracts, when given subcutaneously to mice in very dilute water solution, proved fatal, and the animals exhibited the same symptoms in every case. The ether extract, on evaporating off the solvent, taking up the water, and evaporating to dryness, left a minute quantity of light-yellow gum only slightly soluble in water, from which it separated in concentrated solution in the form of yellow oily globules. A similar material was prepared from the alcohol and water extracts, much the greater quantity being found in alcohol.

³ HILLS, J. S., and WYNNE, W. P. LININ. [London] Chem. Soc. Proc. 21: 74. 1905.
ROBERT, K. DIE ABFÜHRENDE WIRKUNG VON LINUM CATHARTICUM. Pharm. Ztg. 50: 370. 1905.

It seemed to follow from these results—namely, that the poisoned animals exhibited the same symptoms from each of the extracts and the same material could be isolated from all three extracts—that a single poisonous substance was involved which was sparingly soluble in water and in ether, but much more so in alcohol.

PREPARATION OF THE POISON

The dried ground plant was thoroughly extracted with boiling 95 per cent alcohol. The alcohol was distilled from the extract, the residue taken up with water, and the precipitated chlorophyll filtered off. Lead acetate was added to the solution, the resulting precipitate removed by filtration, and the excess of lead with hydrogen sulphide. The aqueous solution thus obtained was concentrated to small bulk on the water bath and then shaken out several times with acetic ether, which removed the active principle. On evaporating the solvent, a light-yellow gummy residue remained, which gradually dried to a hard, brittle, resinous material. By this procedure it was found that 100 gm. of the plant yielded 6.6 gm. of the crude poison, or 6.6 per cent. That the material prepared as described contains the active principle in the plant was proved beyond any doubt by its effect on mice. Given by injection or eaten on bread it proved fatal in every case, with the symptoms as described above.

CHEMICAL PROPERTIES OF THE POISON

The toxic substance is amorphous and has so far resisted efforts to crystallize it. It contains no nitrogen. It reduces Fehling's solution after boiling with dilute hydrochloric acid, but not before; and this property in conjunction with its solubility in acetic ether, suggests the probability that it is a glucoside. No recognizable products resulting from its hydrolysis were found. Likewise, no significant substances were found by either oxidizing or reducing agents. With concentrated nitric acid it gives a brilliant carmine red color which gradually fades to brown. It yields a permanent foam when shaken with water, suggesting a saponin. It dissolves in water to the extent of 3 parts to 100 at 20° C., and is more soluble in hot water. It is very soluble in ethyl or methyl alcohol, but sparingly soluble in most of the other common organic solvents.

In what has preceded the writers have endeavored to show that *Linum neomexicanum* contains a poison of a highly toxic nature, and that it should be classed among plants dangerous to animals. The poison is probably a glucoside and slow in action but fatal in minute doses when administered either subcutaneously or per os. The new substance has been provisionally named "linotoxin."

PHOTOPERIODIC RESPONSE OF SOYBEANS IN RELATION TO TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS¹

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INTRODUCTION

In earlier papers^{2 3} dealing with the effect of relative length of day and night on plant growth and development, considerable attention was given to the contrasted responses of early and late varieties of soybeans to changes in day length. These contrasted responses seemed to be correlated with the changes in behavior of the different varieties under field conditions with advance of the season. Of the four varieties studied under field conditions in the vicinity of Washington, D. C., the Biloxi consistently behaved as a very late variety, earliest plantings first coming into flower about September 1 and failing to mature seed; the Tokyo was somewhat earlier, the first plantings usually flowering early in August and successfully maturing seed; the Peking flowered about 10 days earlier than the Tokyo, or toward the close of July, being well adapted to the region; the Mandarin behaved as an early variety, flowering 3 to 4 weeks after germination. Thus the normal vegetative periods of early plantings of the four varieties, in the order named, are about 95, 65, 55, and 25 days, respectively.

It was found that, when early plantings of the four varieties are exposed to an artificially shortened daylight period of 10 to 12 hours or less, all varieties tend to flower at about the same time, namely, 20 to 25 days after germination. In other words, all behave as early varieties. Again, in field plantings of the four varieties made at intervals of three or four days through a single growing season, the vegetative period of plantings of the Mandarin made during the months of June and July did not change materially; while in the other varieties there was a progressive shortening of the vegetative period as the season advanced. The latest variety, Biloxi, showed the maximum shortening of the preflowering period of growth with advance of the season. Hence, in the late plantings there is, again, an evident tendency in all varieties to behave as when exposed to an artificially shortened day length in early summer. However, the duration of the vegetative period of the late plantings failed to reach the lower level of 20 to 25 days obtained with the shortened day lengths in early summer. Moreover, a considerable lengthening of the vegetative period was observed in the very early plantings of all

¹ Received for publication June 28, 1930, issued November, 1930.

² GARNER, W. W. and ALLARD, H. A. EFFECT OF RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. *Jour. Agr. Research* 18: 553-606, illus. 1920.

³ ——— and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. *Jour. Agr. Research* 23: 871-920, illus. 1923.

varieties, including the Mandarin. The tentative conclusion was reached that under practical conditions in the field the differences in behavior of soybean varieties with respect to time of flowering are due primarily to length of day, while the relatively low temperatures of late spring and early fall exercise a retarding influence on the flowering stage in both the very early and the very late plantings of all varieties.

METHODS

It seemed probable that the relationships existing between the early and late forms of soybeans were more or less applicable to a large group of species of which the different forms normally flower on different dates in early and late summer and early fall. It appeared very desirable, therefore, to obtain more definite data on the inter-relationship of length of day and temperature as influencing the initiation of reproductive activity in the different varieties of soybeans under natural conditions in the field. Such information should be of considerable value in arriving at a conclusion as to the actual significance of length of day as a factor in the natural distribution of plants and their adaptation to different latitudes.

For this purpose three series of experiments were carried out. In the first series, field plantings of the four varieties of soybeans were made at regular intervals of four or five days throughout the growing season at Washington for the years 1920 and 1922 to 1927, inclusive. The results in this series furnish a basis for determining the normal behavior of the different varieties with the advance of the open season in the vicinity of Washington. In the second series of tests, plantings were made in the greenhouse at regular intervals throughout the year and the mean temperature was maintained at an approximately uniform level. In the third series, beginning as early in the spring as outside conditions would permit, plantings of the soybeans were made in boxes in the greenhouse at regular intervals, and as soon as germination had taken place the plants were subjected to a fixed day length of 10 hours under approximately outdoor conditions. To accomplish this the usual arrangement of trucks on steel tracks and ventilated dark houses was employed.⁴ The plants in the first series, of course, were subjected to the natural conditions of day length and temperature prevailing during the open growing season; those in the second series were exposed to the natural change in day length taking place throughout the year, but under a relatively fixed mean temperature approximating that of midsummer; the only essential difference between the treatment given in the third series and that given in the first series was that the plants in the third series were exposed to a constant day length of short duration.

EXPERIMENTAL DATA

FIELD PLANTINGS MADE AT INTERVALS THROUGH THE GROWING SEASON

The plantings were made at intervals of three to five days through the season in accordance with the plan followed in 1919, as described in the writers' first paper.⁵ The tests were carried out each year from

⁴ A description of the outfit used is contained in an earlier publication. See GARNER, W. W., and ALLARD, H. A. *Op. cit.*, p. 559. (See footnote 2.)

⁵ GARNER, W. W., and ALLARD, H. A. *Op. cit.*, p. 569. (See footnote 2.)

1920 to 1927, inclusive, except in 1921. The period required for germination of the seed naturally varied somewhat, depending on prevailing weather conditions, and consequently the dates of germination did not conform rigidly to the schedule of plantings. In order to tabulate the experimental data in summarized form, the actual dates of germination were arbitrarily grouped into periods of one week each and the middle of the week was taken as the average date of germination for a group. For example, all plantings germinating during the week of May 16 to 22 are classed as having germinated on May 19. In some instances an error of one or two days for the averaged dates of germination may be involved, but the results are sufficiently accurate for practical purposes. The earliest date on which flowering had become general in each planting was noted, as was the average height attained by the plants. The results are presented in summarized form in Tables 1, 2, 3, and 4. The data previously obtained in 1919 also are included in the tables. Because of poor seed the Tokyo plantings in 1923 were largely a failure and are not included in the tables.

The average values for the 8-year period are shown graphically in Figure 1. The graph for Biloxi plantings in the greenhouse covering the same period of the year, as described under the next section, is added to facilitate comparison. The seasonal change in length of day, which of course is essentially uniform from year to year, is shown graphically in Figure 2. The average mean temperature during the vegetative period of each planting of the four varieties for the eight years was computed from the Weather Bureau records, and the values applicable to the Mandarin and Peking varieties are shown in Figure 1. The data for Tokyo and Biloxi are very nearly the same as those for Mandarin and Peking and hence are omitted. Naturally, the plantings of late June and early July experienced the highest average temperature (75° to 77° F.) for the preflowering period of growth. In considering these temperature data, as shown in Figure 1, it is to be remembered that the figures do not refer to specific calendar periods indicated in the chart but rather to the periods of time elapsing between germination and first flowering in the particular plantings indicated in each case.

The yearly fluctuations in time of flowering of plantings germinating on any given date are considerable, the extreme range being about 15 days. Also, the heights of the plants vary widely from year to year. Presumably length of day is not a significant factor in these yearly fluctuations, but irregularities in temperature and other variable factors might well account for the observed fluctuations. To afford ready comparison of the variations in length of the vegetative period of the soybeans and differences in the prevailing temperature, the detailed data for the Biloxi and Mandarin varieties, together with the mean temperatures of the vegetative periods in 1925 and 1927, are shown in Figure 3.

TABLE 2.—Dates of germination and flowering, duration of vegetative period, and average heights of the Peking variety of soybeans planted at intervals of three to five days during the open growing season at the Arlington Experiment Farm, Rosslyn, Va., in the years 1919, 1920, and 1922-1927

Average date of germination	Date of first blossoming in—				Duration of vegetative period in—				Average height of plants in—									
	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average
May 19	July 12	July 29	July 18	July 19	July 15	July 13	July 28	July 28	July 21	54	71	60	55	72	63	31	30	24
May 26	July 18	July 30	July 19	July 24	July 19	July 18	July 28	July 28	July 24	53	65	54	66	70	58	30	25	21
June 2	July 24	Aug. 1	July 23	July 27	July 20	July 19	Aug. 1	Aug. 1	Aug. 1	53	60	51	52	65	56	30	27	20
June 9	Aug. 4	Aug. 5	July 29	Aug. 3	Aug. 7	Aug. 2	Aug. 1	Aug. 1	Aug. 1	56	55	47	48	53	49	33	28	17
June 16	Aug. 11	Aug. 5	Aug. 2	Aug. 10	Aug. 7	Aug. 4	Aug. 6	Aug. 6	Aug. 9	54	48	44	45	53	48	36	25	12
June 23	Aug. 18	Aug. 13	Aug. 6	Aug. 14	Aug. 11	Aug. 8	Aug. 11	Aug. 11	Aug. 12	46	44	41	45	44	41	33	20	24
June 30	Aug. 15	Aug. 10	Aug. 10	Aug. 19	Aug. 14	Aug. 13	Aug. 10	Aug. 10	Aug. 10	44	42	39	43	42	40	28	13	22
July 7	Aug. 18	Aug. 18	Aug. 15	Aug. 20	Aug. 18	Aug. 18	Aug. 15	Aug. 15	Aug. 17	42	38	37	42	43	40	25	10	19
July 14	Aug. 21	Aug. 21	Aug. 20	Aug. 23	Aug. 20	Aug. 31	Aug. 30	Aug. 29	Aug. 27	40	36	34	38	40	37	26	11	11
July 21	Aug. 25	Aug. 23	Aug. 26	Aug. 28	Aug. 31	Aug. 30	Sept. 3	Sept. 3	Sept. 2	35	33	30	38	40	37	25	10	19
July 28	Sept. 6	Aug. 31	Sept. 3	Sept. 3	Sept. 8	Sept. 5	Sept. 10	Sept. 10	Sept. 6	34	33	31	36	37	34	27	11	19
Aug. 4	Sept. 13	Sept. 2	Sept. 5	Sept. 6	Sept. 10	Sept. 9	Sept. 13	Sept. 13	Sept. 11	31	29	31	33	34	33	25	10	16
Aug. 11	Sept. 11	Sept. 15	Sept. 15	Sept. 17	Sept. 17	Sept. 15	Sept. 19	Sept. 19	Sept. 18	29	28	29	30	30	29	22	8	14
Aug. 18	Sept. 18	Sept. 18	Sept. 22	Sept. 25	Sept. 27	Sept. 26	Sept. 28	Sept. 28	Sept. 27	31	28	30	31	31	31	22	10	12
Aug. 25	Sept. 26	Sept. 25	Sept. 22	Sept. 28	Sept. 27	Sept. 26	Sept. 29	Sept. 29	Sept. 27	32	31	30	34	36	34	17	9	10
Sept. 1	Sept. 26	Oct. 1	Oct. 1	Oct. 1	Oct. 2	Sept. 29	Oct. 5	Sept. 30	Sept. 30	32	31	30	34	36	34	17	9	10

TABLE 3.—*Dates of germination and flowering, duration of vegetative period, and average heights of the Tokyo variety of soybeans planted at intervals of three to five days during the open growing season at the Arlington Experiment Farm, Rosslyn, Va., in the years 1919, 1920, 1922, and 1924-1927*

Average date of germination	Date of first blossoming in—										Duration of vegetative period in—										Average height of plants in—												
	1919		1920		1922		1923		1924		1925		1926		1927		Average		1919		1920		1922		1923		1924		1925		1926		Average
	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	Days	In	
May 19	July 29	Aug. 1	Aug. 3	July 24	Aug. 11	Aug. 10	Aug. 13	Aug. 15	Aug. 16	Aug. 17	Aug. 18	Aug. 19	Aug. 20	Aug. 21	Aug. 22	Aug. 23	Aug. 24	Aug. 25	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10
May 26	Aug. 1	Aug. 1	Aug. 4	July 28	Aug. 15	Aug. 13	Aug. 16	Aug. 18	Aug. 19	Aug. 20	Aug. 21	Aug. 22	Aug. 23	Aug. 24	Aug. 25	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13
June 2	Aug. 15	Aug. 15	Aug. 9	Aug. 7	Aug. 19	Aug. 16	Aug. 18	Aug. 19	Aug. 20	Aug. 21	Aug. 22	Aug. 23	Aug. 24	Aug. 25	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14
June 9	Aug. 19	Aug. 19	Aug. 11	Aug. 11	Aug. 23	Aug. 20	Aug. 22	Aug. 23	Aug. 24	Aug. 25	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14	Sept. 15	Sept. 16	Sept. 17	
June 16	Aug. 20	Aug. 20	Aug. 16	Aug. 13	Aug. 26	Aug. 23	Aug. 25	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14	Sept. 15	Sept. 16	Sept. 17	Sept. 18	Sept. 19	Sept. 20	
June 23	Aug. 21	Aug. 21	Aug. 19	Aug. 15	Aug. 27	Aug. 24	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14	Sept. 15	Sept. 16	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Sept. 21	
June 30	Aug. 27	Aug. 27	Aug. 22	Aug. 21	Aug. 31	Aug. 28	Aug. 30	Sept. 1	Sept. 2	Sept. 3	Sept. 4	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14	Sept. 15	Sept. 16	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	
July 7	Aug. 31	Aug. 31	Aug. 29	Aug. 24	Sept. 7	Sept. 4	Sept. 6	Sept. 7	Sept. 8	Sept. 9	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14	Sept. 15	Sept. 16	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	
July 14	Sept. 5	Sept. 5	Sept. 1	Sept. 6	Sept. 13	Sept. 11	Sept. 13	Sept. 14	Sept. 15	Sept. 16	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	Oct. 2	Oct. 3	Oct. 4	Oct. 5	Oct. 6	Oct. 7	Oct. 8	
July 21	Sept. 10	Sept. 10	Sept. 8	Sept. 13	Sept. 17	Sept. 15	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	Oct. 2	Oct. 3	Oct. 4	Oct. 5	Oct. 6	Oct. 7	Oct. 8	Oct. 9	Oct. 10	Oct. 11	Oct. 12	
July 28	Sept. 11	Sept. 11	Sept. 9	Sept. 14	Sept. 18	Sept. 16	Sept. 18	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	Oct. 2	Oct. 3	Oct. 4	Oct. 5	Oct. 6	Oct. 7	Oct. 8	Oct. 9	Oct. 10	Oct. 11	Oct. 12	Oct. 13	
Aug. 4	Sept. 11	Sept. 11	Sept. 8	Sept. 14	Sept. 18	Sept. 16	Sept. 18	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	Oct. 2	Oct. 3	Oct. 4	Oct. 5	Oct. 6	Oct. 7	Oct. 8	Oct. 9	Oct. 10	Oct. 11	Oct. 12	Oct. 13	
Aug. 11	Sept. 12	Sept. 12	Sept. 9	Sept. 15	Sept. 19	Sept. 17	Sept. 19	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	Oct. 2	Oct. 3	Oct. 4	Oct. 5	Oct. 6	Oct. 7	Oct. 8	Oct. 9	Oct. 10	Oct. 11	Oct. 12	Oct. 13	Oct. 14	
Aug. 18	Sept. 13	Sept. 13	Sept. 10	Sept. 16	Sept. 20	Sept. 18	Sept. 20	Sept. 21	Sept. 22	Sept. 23	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29	Sept. 30	Oct. 1	Oct. 2	Oct. 3	Oct. 4	Oct. 5	Oct. 6	Oct. 7	Oct. 8	Oct. 9	Oct. 10	Oct. 11	Oct. 12	Oct. 13	Oct. 14	Oct. 15	

The supply of moisture in the soil available to the plant and the relative humidity are additional factors which conceivably might affect the initiation of reproductive processes. For obvious reasons records of rainfall alone do not furnish a very satisfactory indication of available soil moisture, but for the sake of comparison the total rainfall was computed from Weather Bureau records during the period of vegetative growth of each planting of the Mandarin in 1925, 1926, and 1927, and the data are given in Table 5. Corresponding values of the computed relative humidity also are included in the table. The figures for relative humidity were obtained by averaging the

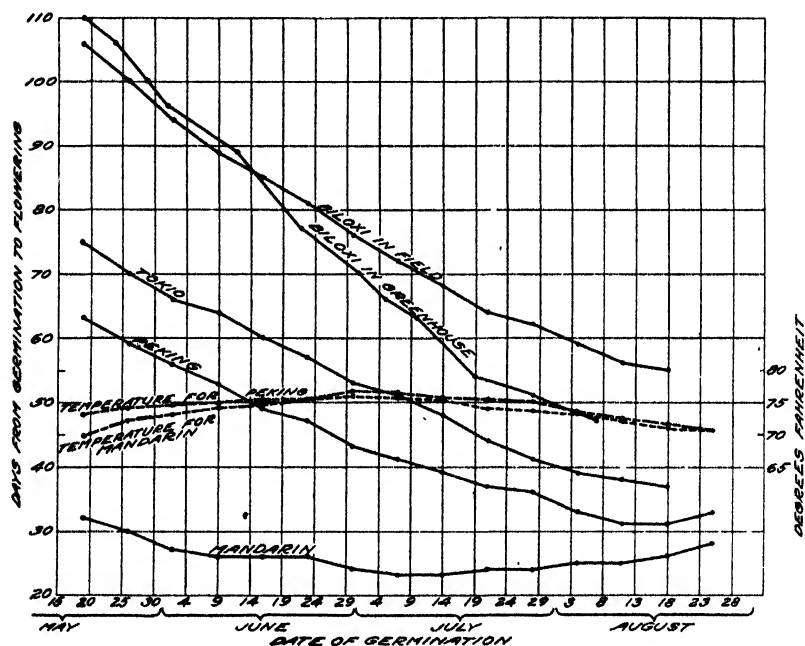


FIGURE 1.—Average number of days from germination to first flowering in field plantings at Arlington Experiment Farm, Rosslyn, Va., of early, medium, and late varieties of soybeans made at short intervals through the growing season in 1919, 1920, and 1922-1927, and in similar greenhouse plantings of the latest variety (Biloxi) in the corresponding season of 1928, also the computed average mean temperature for the vegetative period of each planting of the early (Mandarin) and medium (Peking) varieties. In all except the earliest variety there is a progressive shortening of the vegetative period with advance of season, the maximum effect occurring in the latest variety, Biloxi (see text p. 731)

observations made daily at 8 a. m., noon, and 8 p. m. by the Weather Bureau.

GREENHOUSE PLANTINGS MADE AT INTERVALS THROUGH THE YEAR

Beginning June 18, plantings of Mandarin, Peking, and Biloxi soybeans were made in the greenhouse at intervals of three to five days. The experiment was continued till the middle of August in the following year, a period of about 14 months. An effort was made to maintain an approximately constant mean temperature as far as conditions would permit, but there was no control of the humidity. Facilities were not available for automatic control of the temperature, but it was possible to secure fairly satisfactory results, particularly during

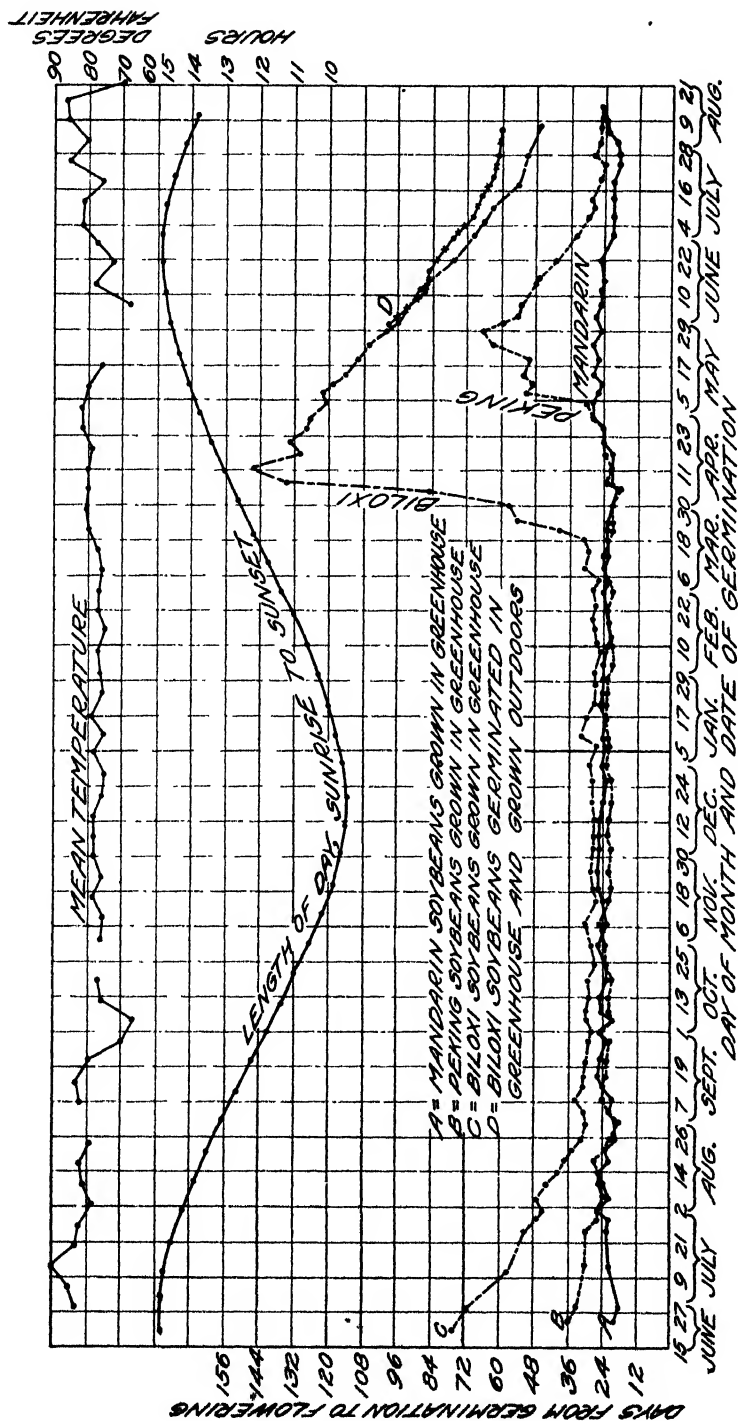


FIGURE 2.—Number of days from germination to first flowering in greenhouse plantings of early, medium, and late varieties of soybeans made at frequent intervals through the year; also the mean weekly temperature in the greenhouse and the seasonal change in length of day. With a favorable, comparatively constant temperature the vegetative period of all varieties was about the same during the fall, winter, and early spring, when the days were short. Later in the spring the increasing day length showed a marked selective action on the different varieties, and only the early variety continued to flower during the longest days of summer (see text, p. 731)

the late fall, winter, and early spring, by hand control of heating and ventilation. Unfortunately, three of the weekly temperature records were lost. In general, the temperature level was close to the mean midsummer temperature at Washington.

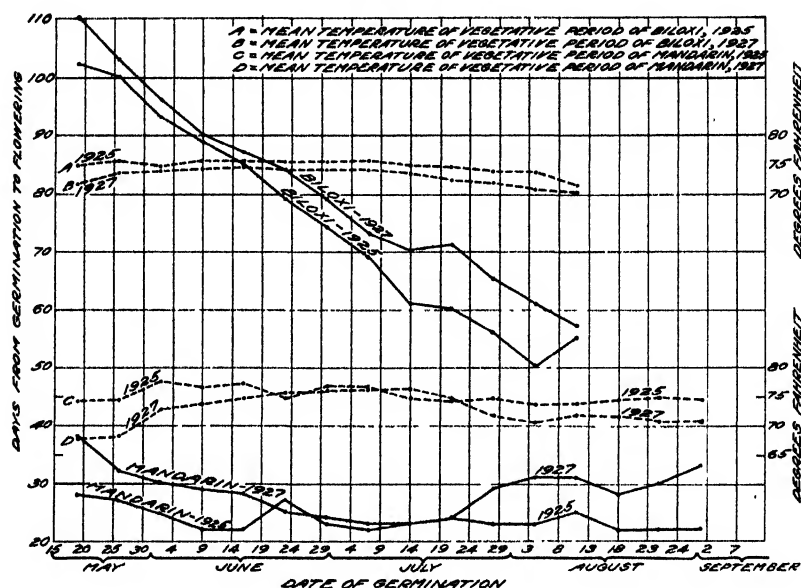


FIGURE 3.—Number of days from germination to first flowering in the successive plantings of the earliest (Mandarin) and the latest (Biloxi) varieties of soybeans in 1925 and 1927, also the computed mean temperature during the vegetative period of each planting of the two varieties. Earlier flowering in 1925 as compared with 1927 in all plantings, except those of the Mandarin made in midsummer, seems to be definitely correlated with the relatively high temperature prevailing in 1925 (see text, p. 730)

TABLE 5.—Mean relative humidity and total rainfall during the preflowering stage of growth in successive field plantings of Mandarin soybeans at Washington, D. C., in 1925, 1926, and 1927

Average date of germination	Mean relative humidity in—			Total rainfall in -		
	1925	1926	1927	1925	1926	1927
	Per cent	Per cent	Per cent	Inches	Inches	Inches
May 19.....	56	59	68	0.77	1.62	3.26
May 26.....	59	60	68	1.22	1.73	3.98
June 2.....	59	61	66	1.51	1.38	3.98
June 9.....	59	64	65	1.90	2.56	4.00
June 16.....	63	64	68	2.61	2.41	2.47
June 23.....	66	64	67	2.46	3.53	5.10
June 30.....	64	66	70	1.86	4.01	1.18
July 7.....	65	65	70	3.36	2.52	1.48
July 14.....	65	69	70	2.92	2.13	2.05
July 21.....	67	69	68	5.42	1.34	1.34
July 28.....	67	75	71	3.85	4.72	3.42
August 4.....	67	76	72	3.24	5.18	4.18
August 11.....	66	79	78	2.52	8.57	3.16

The results with respect to duration of the vegetative period obtained with each variety are shown by means of graphs in Figure 2. The mean weekly temperatures in the greenhouse and the annual range in

day length, sunrise to sunset, are likewise shown in the form of graphs. To facilitate comparison the results with Biloxi for the period of the year corresponding to that of the field plantings are plotted in part also in Figure 3.

In connection with the above tests, plantings of the Biloxi were made in boxes in the greenhouse at intervals, beginning May 27, and after germination had taken place the boxes were placed out of doors. The time required for each planting to reach the flowering stage, as compared with the results in the greenhouse, is shown in Figure 2, D.

PLANTINGS THROUGH THE OPEN GROWING SEASON EXPOSED TO A 10-HOUR DAY

Beginning April 20, plantings of the Peking and Biloxi varieties were made at intervals of three to five days in large boxes in the greenhouse. After germination the boxes were placed on trucks which were daily wheeled out of doors early in the morning and 10 hours later returned to ventilated dark houses having temperatures approximating those prevailing outdoors. In this way the soybeans were

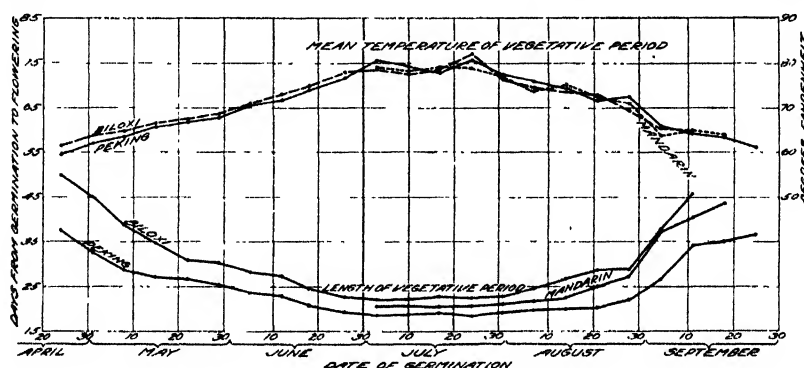


FIGURE 4.—Number of days from germination to first flowering in successive plantings of the Mandarin, Peking, and Biloxi varieties of soybeans which were exposed to approximately outdoor conditions of temperature and a fixed day length of 10 hours. The midsummer temperature appears to be near the optimum for flowering in all varieties, and the lower temperatures of late spring and early fall cause a considerable delay in flowering. There seems to be little indication of a selective action of temperature on the early and late varieties.

exposed to a fixed day length of 10 hours but were subjected to essentially outdoor conditions of temperature. Beginning July 1, similar plantings of the Mandarin variety were included in the test. The results as to time required for each variety to reach the flowering stage are shown graphically in Figure 4, which also shows in each instance the computed mean temperature of the vegetative period of plants.

The Weather Bureau records indicate that for the most part the seasonal weather conditions in the year of the test (1928) were fairly close to normal, especially with respect to temperature. The mean temperature for August, however, was 2 degrees above normal and that of September 3 degrees below normal. Except in July the relative humidity averaged somewhat above normal. The rainfall was below normal in June and July but extraordinarily heavy in August and above normal in September. On the whole the weather conditions seem to have been such as to give a very good picture of the average seasonal effect of temperature on soybeans when the factor of length of day is inoperative.

DISCUSSION OF RESULTS

As a basis for interpreting the results obtained in the present series of experiments, it may be regarded as established that the Mandarin, Peking, Tokyo, and Biloxi varieties of soybeans, which range in behavior from very early to very late flowering types when grown in the field in the vicinity of Washington, D. C., all flower in midsummer within a period of 20 to 25 days after germination, if exposed to an artificially shortened day length. In other words, when exposed to a warm summer temperature and a short day, all behave as early varieties. It has been observed also that when grown in a warm greenhouse in the winter months, when the days are naturally short, these varieties show the same behavior. It has been shown in an earlier paper, moreover, that the soybean is a warmth-loving type, with the result that a cool temperature tends to delay flowering. This fact has been further brought out by Eaton⁶ and by Gilbert.⁷

In the successive plantings in the field through the season, covering a period of eight years (Tables 1, 2, 3, 4, and fig. 1), there are two features of the growth relations which are of interest: (1) Variations from year to year in growth and in date of flowering of plantings germinating on any given date; (2) a general trend toward reduced growth in all varieties and shortening of the vegetative period in all varieties except the Mandarin as the date of germination is advanced. Despite the rather wide variations from year to year, the general trends with advance of season are clearly evident in each year of the test. With respect to the yearly fluctuations, the results in the years 1925 and 1927 (fig. 3) serve to bring out the significance of variation in temperature as a causal factor. In the Biloxi the vegetative period was longer in 1927 than in 1925 throughout the season. In the Mandarin the same difference occurs except in the midsummer plantings. In both cases there is obviously a close correlation between the differences in mean temperature and the differences in length of the preflowering growth period in the two years. Under the conditions of the experiment, temperature seems to be the dominant factor in the yearly fluctuations in time of flowering. The indications are that sustained temperatures below the midsummer average of about 75° to 77° F. will ordinarily tend to cause a delay in flowering. Moreover, it appears that, under the conditions, a decrease of 1° in the mean temperature of the vegetative period causes a delay of some two or three days in date of flowering.

A comparison of the computed mean relative humidity and the total rainfall during the vegetative period of each of the Mandarin plantings in 1925, 1926, and 1927 (Table 5) with the duration of the period of vegetative activity (Table 1) fails to show any consistent correlation. For example, the longer vegetative period of the 1927 plantings germinating prior to June 23, as compared with the 1925 data, is associated with a higher humidity and increased rainfall. When the 1926 data are included in the comparison, however, it is seen that in all plantings germinating prior to June 23 the dates of flowering in 1926 and 1927 agree closely and are considerably later than in 1925, whereas the humidity records and in large part the

⁶ EATON, F. M. ASSIMILATION-RESPIRATION BALANCE AS RELATED TO LENGTH OF DAY REACTIONS OF SOYBEANS. *Bot. Gaz.* 77: 311-321, illus. 1924.

⁷ GILBERT, R. E. THE RESPONSE OF CERTAIN PHOTOPERIODIC PLANTS TO DIFFERING TEMPERATURE AND HUMIDITY. *Ann. Bot. [London]* 40: 315-320, illus. 1925.

rainfall data for 1926 agree rather closely with those of 1925 but not with those of 1927. Again, during the latter part of the summer the relative humidity was much higher in 1926 than in 1925, but there was no decided difference in the duration of the vegetative period in the soybeans.

A comparison of the average growth rates of the soybeans in 1925 and 1927 shows decided differences except in the earlier germinations of the Mandarin and Biloxi (Tables 1-4), and the relative heights attained by the plants were much reduced in 1927. On the whole, correlation of differences in height of the plants with temperature differences is not consistently maintained, indicating that other potent factors also are operative. As an illustration, it will be noted that the materially lower temperature prevailing in 1927 during the growing period of early plantings of the Mandarin and Biloxi (fig. 3) failed to retard the growth as compared with results in 1925. Considering the average results for the eight years, it is evident that the final heights attained are greatest in the early plantings.

Obviously, the irregularities, or fluctuations, in duration of the vegetative phase of growth at any given period of the year will tend to disappear when average results over a period of years are considered. As a consequence, the graphs showing the changes in duration of the vegetative phase in the different varieties with advance of season (fig. 1) become relatively smooth, thus presenting very clearly defined trends. In this case, in relation to date of flowering, we presumably have, besides the day-length factor, the effect of a relatively uniform seasonal trend in temperature, instead of the more or less sharply fluctuating temperature conditions likely to occur in any single year. In all except the very early variety of soybeans the effects of temperature and length of day on late spring and early summer plantings apparently are additive, both the rising temperature and the decreasing day length favoring earlier flowering with advance of season. In midsummer the average temperature seems to be near the optimum, and only the length of day acts as a major limiting factor. As soon after midsummer as the average temperature begins to fall the two factors of day length and temperature become opposed, the former tending to hasten flowering and the latter to delay it. With the very early variety, Mandarin, neither day length nor temperature is an important limiting factor in midsummer, while in spring and early fall temperature is the only primary factor.

The above-stated considerations relate primarily to the behavior of the different varieties, considered individually. It is of special interest, however, to determine to what extent the sharp contrasts in behavior with respect to earliness shown by the different varieties are really due to the factors of day length and temperature. Under field conditions at Washington these varietal distinctions are always clearly apparent despite irregularities in actual time of flowering induced by temperature fluctuations. The greenhouse plantings through the year furnish a clear picture of the relative effects of day length on the duration of the vegetative period of the different varieties at different seasons of the year when the obscuring effects of varying temperature are largely removed. (Fig. 2.) During the 6-month period in which the day length is about 12 hours or less, the distinction between the varieties with respect to earliness is almost entirely lost, a fact which is in accord with conclusions reached in the

earlier work. However, as has been previously observed, the minimum duration of the vegetative phase in Biloxi consistently remained slightly greater than for Mandarin and Peking.

Beginning about March 20, the increasing day length seems to have brought about a rather sudden change in the behavior of the Biloxi. The duration of the vegetative phase of growth increased rapidly till, in the plantings germinating April 4 and shortly thereafter, individual plants in increasing numbers remained in the vegetative stage throughout the summer and till the return of short days in the early fall. After April 17 all individuals in each planting remained in the vegetative stage till fall and for a time the successive plantings tended to flower on the same date. This behavior of the Biloxi with advance of season results in a graph of characteristic form. (Fig. 2.)

A word of caution may not be out of place here relative to the interpretation to be placed upon the portion of the graph showing the lengthening of the vegetative period in the plantings after March 20. It is not to be inferred that these plantings attained the flowering stage in the indicated number of days as a direct result of exposure to the long days of late spring and early summer. On the contrary, it should be understood that flowering was initiated in these plantings only when the excessively long days had shortened to the required point in late summer. There is nothing to indicate how long vegetative activity would have continued if the longest days of summer had remained in effect for a prolonged period. The comparatively uniform decrease in duration of the vegetative period in successive plantings following the planting that germinated April 12 is due to the fact that all the plants began to flower as soon as a fairly definite decrease in day length had taken place. Available information indicates that this occurred early in August.

Beginning about May 8, after the day length had further increased, the Peking practically duplicated the change in behavior shown earlier by the Biloxi except that the change in duration of the vegetative stage of development was somewhat less abrupt and of less magnitude. As in the case of the Biloxi, the distinctive form of this portion of the graph (fig. 2) indicates response to a definite decrease in day length. In contrast with the Biloxi and the Peking, the Mandarin showed no significant change in duration of the vegetative stage at any time during the spring and summer. It is clear that the annual cycle of day length exercises a distinctly selective action on the different varieties of soybeans. Moreover, with respect to earliness in flowering, the relative positions of the three varieties under comparatively constant conditions of temperature remained the same during the period of late spring and summer as in the field plantings previously considered. During the remainder of the year all behaved as early varieties, as previously stated, the preflowering stage of growth being only from 20 to 30 days.

A direct comparison of the behavior of the Biloxi in the greenhouse and in the field is made in Figure 3. It is apparent that in the later plantings the shortening of the vegetative period is less rapid in the field than in the greenhouse, and the explanation probably is to be found in the higher temperature level in the greenhouse. Further evidence of the retarding action of suboptimum temperature on date of flowering is furnished by the Biloxi plantings made in the green-

house and transferred to outdoor conditions of temperature after germination. (Fig. 2, D.)

It remains to consider the effects of change in temperature with advance of season when the length of day is held constant. (Fig. 4.) The fixed day length of 10 hours which was employed is approximately the optimum for flowering in each of the three varieties. As the maximum mean temperature of the vegetative stage of growth is approached in the midsummer plantings, the duration of the vegetative stage attains the minimum value of 18 to 22 days. The generally convex form of the temperature curves constitutes a close counterpart of the concave form of the curves representing the duration of the vegetative period. Evidently there is a definite correlation of temperature with the duration of the vegetative stage. There is, however, no suggestion of the sort of varietal distinction in behavior with advance of season that was associated with the annual cycle in length of day under conditions of constant temperature. Though the evidence is not conclusive, there is some indication that the lower temperatures of late spring and early fall exert a somewhat selective, retarding action on attainment of the flowering stage as between the Mandarin and Biloxi, on the one hand, and the Peking on the other hand. However, the earliest variety, Mandarin, and the latest variety, Biloxi, seemingly respond in much the same way to changes in temperature; and there is no indication of selective action of this factor on these two varieties of soybeans.

So far as may be inferred from the foregoing data, differences in temperature do not account for the differences in behavior of soybean varieties. Seasonal change in length of day exercises a decided selective action on the different varieties and may well constitute the controlling external factor in the differences in time required by the several varieties to attain the flowering stage when grown under similar conditions. The annual range in length of day in the latitude of Washington, from about 9½ to 15 hours, is not sufficient to affect greatly the time of flowering in the very early variety, Mandarin. It is probable, however, that in higher latitudes having a maximum day length of, say, 16 hours or more, this variety would tend to behave about as the Peking does at Washington; that is, the date of flowering would be delayed by the longest days of summer.

In so far as the relationships existing between the early and late forms of soybeans are applicable to the different forms of other warmth-loving species which normally flower in early and late summer and early fall, it appears that the length-of-day factor may exercise a dominant influence on the relative adaptation of these early and late forms to a given latitude. As the sensibility to lower temperature increases, the more essential it becomes that the plant be able to initiate reproductive processes in response to changes in day length occurring comparatively early in the season, if the plant is to reproduce itself successfully in any given region outside the Tropics. This apparently would tend to enable the plant to escape the destructive action of cold. In this connection it may be noted that the progress of the seasonal change in length of day keeps well in advance of the corresponding stage in the seasonal temperature change. Thus, in the latitude of Washington the maximum and the minimum day lengths of the year occur about a month in advance of the maximum and the minimum mean temperatures. It seems logical to suppose

that, in contrast with the interrelationship of temperature and length of day as regards effects on warmth-loving species like soybeans, the drop in temperature as summer gives way to fall will serve to aid the decreasing length of day in hastening the attainment of the reproductive stage in short-day plants of the cool-loving type.

SUMMARY

In this paper consideration is given to the comparative behavior of early, medium, and late varieties of soybeans, more particularly with respect to duration of the purely vegetative stage of development when planted at frequent intervals through the growing season and when grown under partially controlled conditions. When planted early in the season in the vicinity of Washington, D. C., these varieties, known as Mandarin, Peking, Tokyo, and Biloxi, normally require about 25, 55, 65, and 95 days, respectively, for attaining the flowering stage. All varieties are quite sensitive to cool temperatures.

Data were obtained on field plantings made at intervals through the growing season over a period of eight years. The time of flowering of plantings of a given date varied considerably from year to year, and these variations seem to be closely correlated with yearly fluctuations in the prevailing temperature. Definite correlation of fluctuations in date of flowering with differences in relative humidity and rainfall could not be traced, although these factors may not be entirely without effect on the results. Despite the yearly fluctuations, very definite trends as to change in duration of the vegetative stage with advance of the season can be seen in each variety, all except the Mandarin showing progressive shortening of the vegetative stage. The mean midsummer temperature at Washington (75° to 77° F.) appears to be approximately optimum for all varieties and the suboptimum temperatures of spring and late summer tend to delay flowering in the very early and very late plantings.

In plantings of Mandarin, Peking, and Biloxi made in the greenhouse through the year, with a fairly constant mean temperature approximating that of midsummer, there was a period of about six months in which all three varieties reached the flowering stage in about 25 days after germination and, therefore, behaved as early varieties. During this period the length of day ranged approximately from 9½ to 12 hours and it is evident that the short days showed no selective action on the different varieties. Late in March, however, when the day length had increased further, the late-flowering variety, Biloxi, suddenly began to lengthen its period of vegetative growth until a maximum of 146 days was reached or until there was a return to short days in early fall. Beginning about six weeks later in the spring, the Peking experienced the same sort of lengthening of the period of vegetative growth, although the change was less pronounced. The Mandarin showed no decided change in duration of the vegetative phase of development. Thus the increasing length of day exercised a distinct selective action on the three varieties of soybeans, and the contrasts in behavior of these varieties were essentially the same as those obtained in the field. In general, in the later plantings, the progressive shortening of the vegetative period with advance of season was somewhat less pronounced in the field than in the greenhouse, presumably because of the retarding action of the lower

temperature out of doors. Moreover, plantings transferred at germination from the greenhouse to outdoor temperatures showed a delay in flowering.

In plantings of the three varieties made at intervals through the growing season and exposed to a fixed day length of 10 hours, there was apparently a rather close correlation of the length of the pre-flowering stage of growth with the mean temperature. In each variety the minimum vegetative period corresponded to the highest mean temperature of midsummer, with appreciable delays in time of flowering associated with the lower temperatures of late spring and early fall. Apparently the lower temperatures affected the earliest variety, Mandarin, and the latest variety, Biloxi, in much the same way, there being no evidence of a definite selective action of the changing temperature on the two varieties.

The available evidence seems to indicate that under field conditions at Washington variations from year to year in date of flowering of both early and late varieties of soybeans when planted on any particular date are due chiefly to differences in temperature, while length of day is the primary external factor responsible for the fact that one variety is always relatively early and another late in attaining the reproductive stage. It seems likely that the relationships existing between the early and late forms of soybeans will apply equally to many other species, although it does not follow, of course, that the differences in behavior of early and late varieties of all species are explainable on the basis of the length-of-day factor.

The variation in growth rate from year to year in the different varieties, which may be quite marked, seems to be due in part to temperature fluctuations and in part to other environmental factors. Over a period of years the average heights attained by the earlier plantings as a rule markedly exceed those attained by later plantings.

GERMICIDAL EFFICIENCY OF SOAPS AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS¹

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INTRODUCTION

In a previous paper (6)² the writers reported the results of work on the germicidal efficiency of coconut-oil and linseed-oil soaps and of their mixtures with cresol. In the present paper they report the results of similar work done, for the most part, in connection with investigations designed for a different purpose, which will account for the choice of test organisms and some of the other details of the technic.

MATERIALS AND METHODS

The materials used were as follows:

Cochin coconut oil, saponification value, 254.8

Castor oil, saponification value, 179.2.

Linseed oil, saponification value, 191.

Oleic acid, U. S. P., neutralization value, 197.

Lauric acid, C. P., melting point, 43° C.

Phenol, U. S. P., melting point, 40.7° C.

Cresol, U. S. P., boiling point, 195° to 205° C.

Cresylic acid, boiling point, 200° to 240° C. (80 per cent above 205°).

N-Hexylresorcinol, boiling point, 253° to 256° C. at 120 mm. Hg., solidification point, 64° C.

Orthophenylphenol, melting point, 57.5° to 58° C.

Sodium hydroxide, C. P., 96 to 98 per cent NaOH.

The neutral soaps of coconut oil, castor oil, and linseed oil were made by saponifying the respective oils with the required amount of carbonate-free sodium hydroxide solutions in closed flasks on a steam bath. The coconut-oil and castor-oil soaps were then concentrated and cooled and the solid soap was flaked and stored in closed containers. The linseed-oil soap was stored in the liquid form. Sodium oleate was made by adding to a definite weight of oleic acid the chemically equivalent quantity of standard sodium hydroxide solution. The mixture was heated in a closed flask overnight, the flask was allowed to cool to room temperature, and the solution was adjusted to the desired strength by the addition of distilled water. Sodium laurate was made by adding to a definite weight of lauric acid the chemically equivalent quantity of standard sodium hydroxide solution and then adjusting the strength of the solution by adding the proper amount of distilled water.

Alkaline soap solutions were made by adding the desired quantity of sodium hydroxide to the neutral soap solution. The term "added alkali" indicates the presence of sodium hydroxide in excess of that required to form neutral soap.

Acid soap solutions were made by dissolving the fatty acid in the heated stock solution of the neutral soap. The term "added acid"

¹ Received for publication July 24, 1930; issued November, 1930.

Reference is made by number (italic) to Literature Cited, p. 747.

indicates the presence of fatty acid in excess of that required to form neutral soap.

The stock solutions of phenol and soap, and of cresol and soap, were usually concentrated solutions containing approximately 50 per cent of phenol or cresol.

BACTERIOLOGICAL WORK

Bacteriological tests were made by the Rideal-Walker technic, modified as described in the writers' previous paper and still further modified by the use of other test organisms and by the use of beef-infusion broth adjusted to pH 7.2 as the culture medium for all the test organisms except *Eberthella typhi*³ (*B. typhosus*), for which the unadjusted culture medium of Wright was employed. Previous experience having shown that different strains of the same test organism often vary widely in their resistance to disinfectants, tests were made against a number of different strains of each organism. To avoid useless repetition, only results which are considered representative are included in the tables. Where results are given for more than one strain, the purpose is to indicate the variation in resistance of different strains. Results obtained with some of the soaps against *E. typhi* are shown in Table 1.

TABLE 1.—Germicidal efficiency of phenol and of various soaps against *Eberthella typhi*

Disinfectant	Dilution	Added alkali ^a Per cent ^c	Organic matter Per cent	Results after indicated period of exposure (minutes) ^b						Test organism
				2½	5	7½	10	12½	15	
Phenol.....	1-95			+	+	-	-	-	-	E. typhi No. 1
	1-100			+	+	-	-	-	-	
	1-150			+	+	+	-	-	-	
	1-200			+	+	+	-	-	-	
	1-300	50		+	+	-	-	-	-	
	1-400			+	+	-	-	-	-	
Coconut-oil soap.....	1-400			+	+	-	-	-	-	
	1-600	100		+	+	+	+	+	+	
	1-800			+	+	+	+	+	+	
	1-30		Skim milk, 50	+	+	+	+	+	+	
	1-40			+	+	+	+	+	+	E. typhi No. 2
	1-50			+	+	+	+	+	+	
	1-120		Skim milk, 50	+	+	+	+	+	+	
	1-140	100		+	+	+	+	+	+	
	1-160			+	+	+	+	+	+	
Linseed-oil soap.....	1-25			+	+	+	+	+	+	
Sodium oleate.....	1-10			+	+	+	+	+	+	
Castor-oil soap.....	1-20			+	+	+	+	+	+	
	1-40			+	+	+	+	+	+	
Phenol.....	1-95			+	+	+	+	+	+	E. typhi No. 5
	1-30			+	+	+	+	+	+	
Coconut-oil soap.....	1-40			+	+	+	+	+	+	
	1-400			+	+	+	+	+	+	
	1-600	100		+	+	+	+	+	+	
Castor-oil soap.....	1-10			+	+	+	+	+	+	
Phenol.....	1-100			+	+	+	+	+	+	
	1-40			+	+	+	+	+	+	
Coconut-oil soap.....	1-60			+	+	+	+	+	+	
	1-80			+	+	+	+	+	+	
	1-400	100		+	+	+	+	+	+	E. typhi No. 5
Castor-oil soap.....	1-10			+	+	+	+	+	+	

^a Sodium hydroxide in excess of that required to form the soap.

^b Growth, (+), no growth (-).

^c Proportion of added to combined alkali.

³ The bacteriological nomenclature used in this paper is that of the third edition of Bergey's Manual of Determinative Bacteriology (1).

It is evident that strain No. 1 is peculiarly sensitive to the germicidal action of coconut-oil soap. Ordinarily the resistance of *Eberthella typhi* to coconut-oil soap is equivalent to that exhibited by strains No. 2 and No. 5. (Table 1.) In a previous paper (6) when only *Eberthella typhi* No. 1 was used to test the efficiency of coconut-oil soap, results were obtained which the writers now know to be too high for other strains of that organism. The addition of sodium hydroxide to coconut-oil soap solutions increases their germicidal efficiency and tends to flatten out the differences observed when the neutral soap is used. The various strains, including No. 1, differed but slightly in resistance to phenol.

Results obtained with coconut-oil soap against *Staphylococcus aureus* and various hemolytic streptococci are shown in Table 2.

TABLE 2.—*Germicidal efficiency of phenol and of coconut-oil soap against Staphylococcus aureus and hemolytic streptococci*

Disinfectant	Dilution	Added alkali ^a	Results after indicated period of exposure						Test organism
			2 1/2	5	7 1/2	10	12 1/2	15	
		Per cent ^c							
Phenol	1:70	100	+	+	—	—	—	—	<i>S. aureus</i> No. 1
	1:5		+	+	+	+	+	+	
Coconut-oil soap	1:20		+	+	+	+	+	+	
	1:30	100	+	+	+	+	+	+	<i>S. aureus</i> No. 4.
Phenol	1:75		+	+	+	+	+	+	
Coconut-oil soap	1:5		+	+	+	+	+	+	
	1:30	100	+	+	+	+	+	+	Hemolytic streptococcus No. 1
	1:40		+	+	+	+	+	+	
Phenol	1:90		+	+	+	+	+	+	
Coconut-oil soap	1:250	100	+	+	+	+	+	+	Hemolytic streptococcus No. 2
	1:300		+	+	+	+	+	+	
Phenol	1:90		+	+	+	+	+	+	
Coconut-oil soap	1:2,000	100	+	+	+	+	+	+	Hemolytic streptococcus No. 3.
	1:90		+	+	+	+	+	+	
Phenol	1:10		+	+	+	+	+	+	
Coconut-oil soap	1:90	100	+	+	+	+	+	+	Hemolytic streptococcus No. 4.
	1:1,000		+	+	+	+	+	+	
Phenol	1:90		+	+	+	+	+	+	
Coconut-oil soap	1:500	100	+	+	+	+	+	+	Hemolytic streptococcus No. 5.
			+	+	+	+	+	+	
			+	+	+	+	+	+	

^a Sodium hydroxide in excess of that required to form the soap.

^b Growth (+), no growth (—).

^c Proportion of added to combined alkali.

Coconut-oil soap in 20 per cent solution did not kill *Staphylococcus aureus* in 15 minutes, but a 5 per cent solution with the addition of 100 per cent excess NaOH killed strain No. 1, the most resistant of the four strains employed, in 7 1/2 minutes.

The results with five strains of streptococci show great differences in resistance to the germicidal action of coconut-oil soap. Somewhat similar variations in the resistance of different strains of streptococci to sodium ricinoleate have been reported by Kozlowski (3).

Results obtained with *Salmonella pullorum* and *S. gallinarum* (*Eberthella sanguinaria*) are shown in Table 3.

The results indicate that coconut-oil soap has approximately the same germicidal efficiency against these organisms as against *Eberthella typhi*. Strain 628 of *Salmonella pullorum* was no longer available when the tests were made with castor-oil soap, but the result shown for strain 786 was confirmed by results obtained with three other strains.

TABLE 3.—Germicidal efficiency of phenol and of various soaps against *Salmonella pullorum* and *S. gallinarum*

Disinfectant	Dilution	Added alkali ^a	Organic matter	Results after indicated period of exposure (minutes) ^b						Test organism
				2½	5	7½	10	12½	15	
		<i>Per ct.^c</i>	<i>Per cent</i>							
Phenol.....	1-90			+	+	+	-	-	-	<i>S. pullorum</i> No. 628.
	1-20			+	+	+	+	+	+	
	1-30			+	+	+	+	+	+	
	1-500			+	+	+	+	+	+	
Coconut-oil soap	1-750			+	+	+	+	+	+	
	1-100			+	+	+	+	+	+	
	1-150	100	Skim milk, 50	+	+	+	+	+	+	<i>S. pullorum</i> No. 786.
	1-50			+	+	+	+	+	+	
	1-100		Feces ^d	+	+	+	+	+	+	
	1-100			+	+	+	+	+	+	
Phenol.....	1-90			+	+	+	-	-	-	
	1-10			+	+	+	+	+	+	<i>S. pullorum</i> No. 786.
Coconut-oil soap.....	1-20			+	+	+	+	+	+	
	1-500			+	+	+	+	+	+	
	1-750	100		+	+	+	+	+	+	
Castor-oil soap.....	1-10			+	+	+	+	+	+	<i>S. gallinarum</i> .
Phenol.....	1-100			+	+	+	+	+	+	
	1-30			+	+	+	+	+	+	
	1-40			+	+	+	+	+	+	
Coconut-oil soap.....	1-400			+	+	+	+	+	+	
	1-600			+	+	+	+	+	+	
	1-100	100	Skim milk, 50.	+	+	+	+	+	+	
	1-120			+	+	+	+	-	-	

^a Sodium hydroxide in excess of that required to form the soap.^b Growth (+), no growth (-).^c Proportion of added to combined alkali.^d 1 gm. dry chicken feces to 5 c. c. of disinfectant.

In Table 4 are shown the results of tests against *Pasteurella arvicola* and *P. suisepctica*, indicating that coconut-oil soap has a very high germicidal efficiency against these organisms in the absence of organic matter.

TABLE 4.—Germicidal efficiency of phenol and of various soaps against *Pasteurella arvicola* and *P. suisepctica*

Disinfectant	Dilution	Added alkali ^a	Organic matter	Results after indicated period of exposure (minutes) ^b						Test organism
				2½	5	7½	10	12½	15	
		<i>Per ct.^c</i>	<i>Per cent</i>							
Phenol.....	1-160			+	+	+	-	-	-	<i>P. arvicola</i> No. 1176.
	1-5000			+	+	+	+	+	+	
	1-50			+	+	+	+	+	+	
	1-100		Skim milk, 50	+	+	+	+	+	+	
Coconut-oil soap.....	1-1,000			+	+	+	+	+	+	
	1-1,200	100	Skim milk, 50	+	+	+	+	+	+	
	1-150			+	+	+	+	+	+	<i>P. arvicola</i> No. 930.
	1-200			+	+	+	+	+	+	
Phenol.....	1-170			+	+	+	+	+	+	
	1-6,000			+	+	+	+	+	+	
	1-8,000			+	+	+	+	+	+	<i>P. arvicola</i> No. 930.
Coconut-oil soap.....	1-2,000			+	+	+	+	+	+	
	1-4,000	10		+	+	+	+	+	+	
	1-1,200	100		+	+	+	+	+	+	
	1-1,400			+	+	+	+	+	+	<i>P. arvicola</i> No. 1.
Coconut-oil soap.....	1-2,000			+	+	+	+	+	+	
	1-1,250			+	+	+	+	+	+	
Castor-oil soap.....	1-1,500			+	+	+	+	+	+	<i>P. suisepctica</i> No. 13.
Linseed-oil soap.....	1-20			+	+	+	+	+	+	
	1-40			+	+	+	+	+	+	
Phenol.....	1-170			+	+	+	+	+	+	
	1-6,000			+	+	+	+	+	+	<i>P. suisepctica</i> No. 37.
Coconut-oil soap.....	1-8,000			+	+	+	+	+	+	
	1-1,200	100		+	+	+	+	+	+	
	1-1,600			+	+	+	+	+	+	
Phenol.....	1-170			+	+	+	+	+	+	<i>P. suisepctica</i> No. 37.
	1-4,000			+	+	+	+	+	+	
Coconut-oil soap.....	1-6,000			+	+	+	+	+	+	
	1-1,000	100		+	+	+	+	+	+	
	1-1,200			+	+	+	+	+	+	

^a Sodium hydroxide in excess of that required to form the soap.^b Growth (+), no growth (-).^c Proportion of added to combined alkali.

The addition of excess sodium hydroxide diminished the efficiency of coconut-oil soap. A decrease in efficiency was caused by as little as 10 per cent excess sodium hydroxide. Organic matter in the form of skim milk also reduced the efficiency of the soap, but this decrease was less when excess NaOH was used. Strain No. 1 is exceptional in its resistance. The limits of variation in resistance of the other seven strains are indicated by the results shown for strains 1176 and 930. It will be seen that when tested against strain No. 1, castor-oil soap had about one-half the germicidal efficiency of coconut-oil soap.

The results shown in Table 5 indicate that the addition of excess lauric acid to sodium laurate increases its germicidal efficiency against *Pasteurella arida* and a hemolytic streptococcus while excess alkali decreases efficiency. As sodium laurate is the principal constituent of coconut-oil soap these results should be considered in connection with the results shown in Table 4.

TABLE 5.—*Germicidal efficiency of neutral, acid, and alkaline sodium laurate against Pasteurella arida and hemolytic streptococcus*

Sodium laurate	Added acid or alkali ^a	Results after indicated period of exposure (minutes) ^b						Test organism
		2½	5	7½	10	12½	15	
	Per cent ^c							
1-6,000		+	+	+	+	+	+	<i>P. arida</i> No. 3.
1-7,000		+	+	+	+	+	+	
1-8,000	Acid, 20.	+	+	+	+	+	+	
1-10,000		+	+	+	+	+	+	
1-1,000	Alkali, 100	+	+	+	+	+	+	
1-1,250		+	+	+	+	+	+	
1-1,500		+	+	+	+	+	+	
1-8,000		+	+	+	+	+	+	
1-9,000		+	+	+	+	+	+	<i>P. arida</i> No. 930
1-10,000	Acid, 10.	+	+	+	+	+	+	
1-12,000		+	+	+	+	+	+	
1-12,000	Acid, 20.	+	+	+	+	+	+	
1-14,000		+	+	+	+	+	+	
1-10,000	Acid, 40.	+	+	+	+	+	+	
1-12,000		+	+	+	+	+	+	
1-2,000		+	+	+	+	+	+	Hemolytic streptococcus No. 4
1-2,400		+	+	+	+	+	+	
1-1,000	Alkali, 100	+	+	+	+	+	+	
1-2,500	Acid, 50	+	+	+	+	+	+	
1-3,000		+	+	+	+	+	+	

^a Lauric acid or sodium hydroxide in excess of that required to form sodium laurate

^b Growth (+), no growth (—)

^c Proportion of added to combined acid or alkali.

The effect of the addition of soap on the germicidal efficiency of phenol is indicated by the results shown in Table 6.

As previous work had indicated probable maximum efficiency with 2 parts of phenol to 1 part of soap, this proportion was used. It will be noted that with *Eberthella typhi* and *Salmonella pullorum* as the test organisms the addition of coconut-oil soap or castor-oil soap multiplied the efficiency of phenol from two to three times while linseed-oil soap increased the efficiency only about 50 per cent. With *Staphylococcus aureus* as the test organism the increase in efficiency was about 50 per cent with each of the three soaps. With *E. typhi* as the test organism sodium oleate did not increase the germicidal efficiency of phenol but on the other hand did not decrease it.

TABLE 6.—Germicidal efficiency of mixtures containing phenol and various soaps

Phenol dilution	Soap ^a	Organic matter	Results after indicated period of exposure, (minutes) ^b						Test organism
			2½	5	7½	10	12½	15	
		Per cent							
1-70			+	+	-	-	-	-	Staphylococcus aureus No. 209
1-100	Coconut-oil...		+	+	+	+	+	+	
1-120			+	+	+	+	+	+	
1-100	Linseed-oil		+	+	+	+	+	+	
1-120			+	+	+	+	+	+	Salmonella pullorum No. 685.
1-120	Castor-oil.....		+	+	+	+	+	+	
1-140			+	+	+	+	+	+	
1-90			+	+	+	+	+	+	Eberthella typhi No. 1.
1-320	Coconut-oil.....		+	+	+	+	+	+	
1-400			+	+	+	+	+	+	
1-240	Castor-oil		+	+	+	+	+	+	
1-320			+	+	+	+	+	+	E. typhi No. 2.
1-95	Coconut-oil..		+	+	+	+	+	+	
1-300			+	+	+	+	+	+	
1-400	Linseed-oil ..		+	+	+	+	+	+	E. typhi No. 1.
1-240			+	+	+	+	+	+	
1-320	Castor-oil		+	+	+	+	+	+	
1-160			+	+	+	+	+	+	E. typhi No. 2.
1-240	Sodium oleate ..		+	+	+	+	+	+	
1-320			+	+	+	+	+	+	E. typhi No. 1.
1-100	None ..	Skim milk, 25.	+	+	+	+	+	+	
1-95		Skim milk, 50.	+	+	+	+	+	+	E. typhi No. 1.
1-85			+	+	+	+	+	+	
1-90			+	+	+	+	+	+	E. typhi No. 1.
1-120	Castor-oil	Skim milk, 50.	+	+	+	+	+	+	
1-160			+	+	+	+	+	+	
1-200			+	+	+	+	+	+	

^a Concentration of soap is half that of phenol.^b (Growth +), no growth (-).

In view of the work of Frobisher (2) showing that sodium oleate decreased the germicidal efficiency of phenol, further experiments were made, the results of which are shown in Table 7.

TABLE 7.—Germicidal efficiency of mixtures of phenol with sodium oleate against *Eberthella typhi*EXPERIMENT 1 ^a

Phenol Dilution	Sodium oleate dilution	Results after indicated period of exposure (minutes) ^b						Test organism
		2½	5	7½	10	12½	15	
1-100		+	+	+	-	-	-	E. typhi No. 1.
1-60	1-30	+	+	+	-	-	-	
1-80	1-40	+	+	+	-	-	-	
1-100	1-50	+	+	+	-	-	-	
1-60	1-15	+	+	+	-	-	-	
1-80	1-20	+	+	+	-	-	-	
1-100	1-25	+	+	+	-	-	-	

EXPERIMENT 2 ^c

		5	10	15	20	30		
^d 1-90		+	+	-	-	-		E. typhi No. 2.
^d 1-100		+	+	-	-	-		
^d 1-110		+	+	-	-	-		
1-100		+	+	-	-	-		
1-110	½ c. c. 1 per cent solution in each tube.	+	+	-	-	-		
1-120		+	+	-	-	-		
1-130		+	+	-	-	-		

^a Medication temperature 25° C., 0.5 c. c. culture to 5 c. c. disinfectant.^b Growth (+), no growth (-).^c Medication temperature 20° C., 0.1 c. c. culture to 5 c. c. disinfectant.^d 0.5 c. c. additional water in each tube.

In experiment 1 the writers' usual technic was employed, while in experiment 2 an attempt was made to approximate the technic described by Frobisher. The amount of culture was 0.1 to 5 c. c. of disinfectant, the medication temperature 20° C., and the test organism the Hopkins strain of *Eberthella typhi*. It should be noted that in experiment 1 sodium oleate was used in the proportions of twice and four times the amount of phenol.

The results presented in Table 8 indicate that the addition of soaps to higher members of the phenol series increases their germicidal efficiency. In the presence of organic matter (Tables 6 and 8) the effect of soaps upon the germicidal efficiency of phenols is greatly diminished.

TABLE 8.—*Germicidal efficiency of mixtures of soaps with higher phenols against various organisms*

Disinfectant		Dilution of phenols	Organic matter	Results after period of exposure (minutes) ^b						Test organism
Phenols	Soap ^a			2½	5	7½	10	12½	15	
Cresol.....		1-240	Per cent	+	-	-	-	-	-	Salmonella pullorum No 685.
		1-280	-----	+	+	+	+	-	-	
		1-320	-----	+	+	+	-	-	-	
		1-400	-----	+	+	+	-	-	-	
		1-600	-----	+	+	+	-	-	-	
		1-700	-----	+	+	+	+	-	-	
		1-700	-----	+	+	+	+	-	-	
		1-800	-----	+	+	+	+	+	+	
		1-180	-----	+	+	+	+	+	+	
		1-200	-----	+	+	+	+	+	+	
		1-240	-----	+	+	+	+	+	+	
		1-280	-----	+	+	+	+	+	+	
		1-300	-----	+	+	+	+	+	+	
		1-350	-----	+	+	+	+	+	+	
		1-350	-----	+	+	+	+	+	+	
		1-350	-----	+	+	+	+	+	+	
		1-280	-----	+	+	+	+	+	+	
		1-300	-----	+	+	+	+	+	+	
		1-400	-----	+	+	+	+	+	+	
		1-480	-----	+	+	+	+	+	+	
		1-700	-----	+	+	+	+	+	+	
		1-800	-----	+	+	+	+	+	+	
		1-700	-----	+	+	+	+	+	+	
		1-800	-----	+	+	+	+	+	+	
None.....		1-220	Skim milk, 50.	+	+	+	+	+	+	Eberthella typhi No. 1.
		1-240		+	+	+	+	+	+	
		1-260		+	+	+	+	+	+	
		1-220	Skim milk, 25.	+	+	+	+	+	+	
		1-240		+	+	+	+	+	+	
		1-260		+	+	+	+	+	+	
		1-240	Skim milk, 50.	+	+	+	+	+	+	
		1-280		+	+	+	+	+	+	
		1-320		+	+	+	+	+	+	
		1-240	Skim milk, 25.	+	+	+	+	+	+	
		1-280		+	+	+	+	+	+	
		1-320		+	+	+	+	+	+	
Linseed-oil ..		1-600	-----	+	+	+	+	+	+	
		1-700	-----	+	+	+	+	+	+	
		1-600	-----	+	+	+	+	+	+	
		1-700	-----	+	+	+	+	+	+	
		1-1100	-----	+	+	+	+	+	+	
		1-1200	-----	+	+	+	+	+	+	
		1-400	Serum, 50.	+	+	+	+	+	+	
		1-480		+	+	+	+	+	+	
		1-400		+	+	+	+	+	+	
		1-480	Serum, 25.	+	+	+	+	+	+	
		1-400		+	+	+	+	+	+	
		1-480		+	+	+	+	+	+	
Coconut-oil....		1-400	Serum, 50.	+	+	+	+	+	+	
		1-480		+	+	+	+	+	+	
		1-480		+	+	+	+	+	+	
		1-560	Serum, 25.	+	+	+	+	+	+	
		1-640		+	+	+	+	+	+	
		1-640		+	+	+	+	+	+	

^a Concentration of soap half that of phenols.

^b Growth (+), no growth (-).

The results of similar experiments with hexylresorcinol are shown in Table 9.

TABLE 9.—*Germicidal efficiency of mixtures of hexylresorcinol with various soaps*

EXPERIMENT 1

Soap	Ratio, soap to hexyl- resorcinol	Hexyl- resorcinol dilution	Results after indicated period of exposure (minutes) *						Test organism
			2½	5	7½	10	12½	15	
None	{	1-10,000	—	—	—	—	—	—	Eberthella typhi No. 1.
		1-12,000	+	+	—	—	—	—	
		1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
Castor-oil	{	1-16,000	+	+	+	+	+	+	
		1-18,000	+	+	—	+	+	+	
		1-12,000	+	+	—	+	—	—	
		1-16,000	+	+	+	+	—	—	
	{	1-12,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
		1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
Coconut-oil	{	1-18,000	+	+	+	+	+	+	
		1-12,000	+	+	—	—	—	—	
		1-16,000	+	+	—	—	—	—	
		1-12,000	+	+	+	+	+	+	

EXPERIMENT 2

None		1-12,000	—	—	—	—	—	—	E. typhi No. 1.
		1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
		1-14,000	+	+	+	+	—	—	
	1:2	1-16,000	+	+	+	+	—	—	
		1-18,000	+	+	+	+	—	—	
		1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
Castor-oil	2:1	1-16,000	+	+	+	+	—	—	
		1-18,000	+	+	+	+	—	—	
		1-10,000	+	+	+	+	—	—	
		1-12,000	+	+	+	+	—	—	
	8:1	1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
		1-18,000	+	+	+	+	—	—	
		1-12,000	+	+	+	+	—	—	
	1:2	1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
		1-18,000	+	+	+	+	—	—	
		1-12,000	+	+	+	+	—	—	
Sodium oleate	2:1	1-14,000	+	+	+	+	—	—	
		1-16,000	+	+	+	+	—	—	
		1-9,000	+	+	+	+	—	—	
		1-12,000	+	+	+	+	—	—	
	4:1	1-15,000	+	+	+	+	+	+	
		1-6,000	+	+	+	+	+	+	
None		1-4,750	—	—	—	—	—	—	E. typhi No. 2.
		1-5,000	+	+	+	+	+	+	
		1-5,250	+	+	+	+	+	+	
		1-5,250	+	+	+	+	+	+	
Coconut-oil	1:2	1-5,500	+	+	+	+	+	+	
		1-5,750	+	+	+	+	+	+	
		1-6,000	+	+	+	+	+	+	
		1-5,500	+	+	+	+	+	+	
Castor-oil	1:2	1-6,000	+	+	+	+	+	+	
		1-5,500	+	+	+	+	+	+	
		1-6,000	+	+	+	+	+	+	
		1-5,000	+	+	+	+	+	+	
None		1-5,500	+	+	+	+	+	+	Staphylococcus aureus No. 200.
		1-6,000	+	+	+	+	+	+	
		1-6,000	+	+	+	+	+	+	
		1-8,000	+	+	+	+	+	+	
Castor-oil	1:2	1-6,000	+	+	+	+	+	+	
		1-8,000	+	+	+	+	+	+	
		1-6,000	+	+	+	+	+	+	
		1-8,000	+	+	+	+	+	+	

* Growth (+), no growth (—).

It should be noted that the stock solution of hexylresorcinol used in experiment 2 differed from that used in experiment 1, and comparisons, therefore, should be made only between mixtures prepared from the same stock solution. Castor-oil soap and coconut-oil soap

used in proportions of 1 part of soap to 2 parts of hexylresorcinol and 2 parts of soap to 1 of hexylresorcinol increased the germicidal efficiency of hexylresorcinol. Used in the same proportions sodium oleate did not have this effect. On the contrary, when larger proportions of sodium oleate were used the efficiency decreased. The marked difference in the resistance of strains No. 1 and No. 2 of *Eberthella typhi* to hexylresorcinol has been reported by the writers in a previous paper (5).

In Table 10 are shown the results obtained with a number of experimental "germicidal" soaps which were made by adding various phenolic compounds to coconut-oil soap. No. 1 contained 25 per cent of cresol with 50 per cent of soap; No. 2 contained 25 per cent of "cresylic acid" with 50 per cent of soap; No. 3 contained 37.5 per cent of cresol with 37.5 per cent of soap; No. 4 contained 37.5 per cent of "cresylic acid" with 37.5 per cent of soap; No. 5 contained 6 per cent of orthophenylphenol with 24 per cent of soap; and No. 6 contained 9 per cent of orthophenylphenol with 18 per cent of soap. Nos. 1 and 2 were solid, Nos. 3 and 4 were soft pastes, and Nos. 5 and 6 were liquid. Soaps numbered 1, 2, 3, and 4 had a distinct phenolic odor while Nos. 5 and 6 were odorless.

TABLE 10.—*Efficiency of experimental germicidal soaps*

Disinfectant	Dilution	Organic matter	Results after indicated period of exposure (minutes) ^a						Test organism
			2½	5	7½	10	12½	15	
		<i>Per cent</i>							
Cresol.....	1:175	}	+	+	+	+	+	+	Staphylococcus aureus No. 209.
	1:200		+	+	+	+	+	+	
	1:125		+	+	+	+	+	+	
Soap No. 1	1:167	}	+	+	+	+	+	+	
	1:25		+	+	+	+	+	+	
	1:125		+	+	+	+	+	+	
Soap No. 2	1:167	}	+	+	+	+	+	+	
	1:25		+	+	+	+	+	+	
	1:50		+	+	+	+	+	+	
Soap No. 3	1:100	}	+	+	+	+	+	+	
	1:50		+	+	+	+	+	+	
	1:100		+	+	+	+	+	+	
Soap No. 4	1:100	}	+	+	+	+	+	+	
	1:100		+	+	+	+	+	+	
	1:100		+	+	+	+	+	+	
Soap No. 5	1:6	}	+	+	+	+	+	+	
	1:12		+	+	+	+	+	+	
	1:6		+	+	+	+	+	+	
Soap No. 6	1:12	}	+	+	+	+	+	+	
	1:18		+	+	+	+	+	+	
	1:36		+	+	+	+	+	+	
Orthophenylphenol ^b	1:18	}	+	+	+	+	+	+	
	1:36		+	+	+	+	+	+	
	1:4,000	}	+	+	+	+	+	+	
	1:6,000		+	+	+	+	+	+	
	1:8,000		+	+	+	+	+	+	
	1:800	}	+	+	+	+	+	Eberthella typhi No. 1.	
	1:1,200		+	+	+	+	+		+

^a Growth (+), no growth (—).

^b Dissolved in 8 parts of coconut-oil soap, dilution expressed in terms of orthophenylphenol.

The orthophenylphenol used in making soaps Nos. 5 and 6 was a high-grade commercial product. A later sample had a very distinct odor due to impurities present, and it would seem that only the higher grade is suitable for the production of an odorless germicidal soap. Results with *Eberthella typhi* in the absence of organic matter

are included in the table in order to indicate the relative value of orthophenylphenol as compared with other disinfectants under the usual conditions of the phenol coefficient test. In view of results already obtained it would seem that the odorless germicidal soap containing orthophenylphenol should prove useful.

DISCUSSION

So far as neutral soaps are concerned, the results presented in this paper confirm and add to those reported by Walker (7). They indicate that coconut-oil soap is the only common soap with significant germicidal activity at room temperature. Against most of the organisms used in this work the germicidal activity of neutral coconut-oil soap was comparatively low even in the absence of organic matter. Against *Pasteurella avicida*, *P. suisepctica*, and some strains of hemolytic streptococci it manifested very high germicidal activity in the absence of organic matter but comparatively low activity in the presence of organic matter in the form of skim milk. It would seem, therefore, that neutral coconut-oil soap is not suitable for the purpose of general disinfection.

In the presence of organic matter coconut-oil soaps containing excess alkali were more active and more uniform in their action than the neutral soaps against the various bacteria tested.

No entirely satisfactory explanation of the germicidal activity of soaps has been worked out as yet. Various writers have attributed it to alkali set free by hydrolysis or to free alkali and unchanged soap combined. Results presented in this paper suggest that while some such explanation may be true for some of the test organisms used in this work, it is not true for all. For bacteria of the *Pasteurella* group the writers' results indicate that the fatty acid plays an important part. According to McBain (4) fatty acid set free by hydrolysis combines with the soap to form acid soap, so it would appear to be the acid soap rather than the free fatty acid which has such high germicidal activity against bacteria of the *Pasteurella* group.

Just as there is no satisfactory explanation for the germicidal activity of soaps so also there is no satisfactory explanation of their action in enhancing the germicidal activity of phenols. Considered from the practical point of view, however, it is plain enough that if a soap is to be used with a phenol it is important to use the right soap and the correct proportion. Results presented in this paper indicate that coconut-oil soap and castor-oil soap are suitable for such use in the proportion of 1 part of soap to 2 parts of phenol.

SUMMARY

Bacteriological tests were made with coconut-oil soap, linseed-oil soap, and castor-oil soap, and mixtures of these with sodium hydroxide or with phenols, against *Eberthella typhi*, *Staphylococcus aureus*, hemolytic streptococci, *Salmonella pullorum*, *Pasteurella avicida*, *P. suisepctica*, and *Salmonella gallinarum*. Some tests were also made with sodium laurate and sodium oleate.

Neutral coconut-oil soap in a concentration of 10 per cent was effective against all the test organisms except *Staphylococcus aureus*. A 20 per cent solution failed to kill *S. aureus* in one hour, while on the

other hand *Pasteurella aricida*, *P. suisepctica*, and some strains of hemolytic streptococci were killed by concentrations varying from 1-1,000 to 1-6,000 within 15 minutes in the absence of organic matter. In the presence of 50 per cent skim milk a concentration of 1-100 was required. Against *P. aricida* neutral linseed-oil soap was effective in 5 per cent concentration, while neutral castor-oil soap was effective in a concentration of 1-1,250, in both instances without organic matter. These two soaps were not tested against *P. suisepctica* but against the other test organisms they were not effective in 10 per cent concentration with 15 minutes exposure.

The addition of sodium hydroxide to solutions of neutral coconut-oil soap made these solutions more effective against all of the test organisms except *Pasteurella aricida*, *P. suisepctica* and some strains of hemolytic streptococci, in which cases efficiency was diminished. The presence of excess alkali decreased the efficiency of sodium laurate against *P. aricida*, while excess lauric acid increased it.

Coconut-oil soap, castor-oil soap, or linseed-oil soap when mixed with various phenolic compounds in the proportion of 1 part of soap to 2 parts of phenol considerably increased the germicidal efficiency of the phenols in the absence of organic matter. In the presence of milk or blood serum such increases were comparatively small.

Germicidal soaps made by adding cresol, commercial cresylic acid, or orthophenylphenol to coconut-oil soap were found to be efficient against *Staphylococcus aureus*, even in the presence of milk or blood serum. The soap containing high-grade orthophenylphenol was odorless.

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VITAMINS IN SUGARCANE JUICE AND IN SOME CANE-JUICE PRODUCTS¹

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INTRODUCTION

In addition to the commonly accepted food value of sugarcane sirup and molasses as carbohydrates and their place in supplying characteristic flavors and variety in the diet, it was formerly believed in certain sections where cane sugar was manufactured that the juice of the cane was particularly healthful and even had some remedial properties. In the early days of the sugar industry in this country the sugar mill was regarded as a very healthful place to work. The evaporation of the cane juice was carried out in large open cauldrons or kettles, and the steam and vapors escaped into the building. The breathing of these vapors was considered beneficial. Large quantities of the cane juice were drunk, and other products of the sugar factory were included in the diet. Whether the reported beneficial results were real or fancied, however, they contribute an added interest to the study of the vitamin content of sugarcane juice.³

As far as the authors are aware but little work has been done on the vitamin content of fresh sugarcane juice. Delf⁴ found that sugarcane juice had no antiscorbutic value.

In this paper are presented the results of some experiments carried out to determine the vitamin B⁵ and vitamin D potency of the juice of sugarcane. Some preliminary tests were also made for vitamin A. The juice used was obtained from three lots of sugarcane stalks shipped at widely different times and from different sections of the country.

In addition to estimating the vitamins in the juice obtained by crushing whole cane stalks in a laboratory-size sugarcane mill, a comparison was made of the vitamin B value of the juice thus expressed with that obtained by subjecting the bagasse from the small mill to the much higher pressure of a hydraulic press. Comparison was also made of the vitamin B potency of juice obtained from different parts of the same cane stalks. For this purpose juice

¹ Received for publication July 16, 1930, issued November, 1930. This paper was presented in part before the American Chemical Society at the seventy-eighth meeting held in Minneapolis, Minn., Sept. 9 to 13, 1929.

² The authors wish to acknowledge their indebtedness to H. S. Paine and R. T. Balch, of the Carbohydrate Division of this bureau, for the analyses of the samples of the cane juice used in this investigation and for supplying the samples of the cane-juice products studied, also to J. F. Brewster for having some of the sugarcane collected and shipped, and to J. P. Divine for technical assistance in carrying out the experimental work.

³ The writers are indebted for the above information to C. A. Browne, at whose suggestion the work described in this paper was undertaken.

⁴ DELF, E. M. STUDIES IN EXPERIMENTAL SCURVY, WITH SPECIAL REFERENCE TO THE ANTISCORBUTIC PROPERTIES OF SOME SOUTH AFRICAN FOODSTUFFS. *Lancet* 202 576-578. 1922.

⁵ Inasmuch as some of the data presented in this paper were obtained before the multiple nature of the water-soluble vitamin B fraction was recognized, the term "vitamin B" has been used in the old sense to refer to the vitamin B complex.

obtained from the upper third and lower third portions of the cane was used. Several products manufactured from sugarcane juice were also examined. These included cane sirup, different samples of blackstrap molasses, and cane cream.

EXPERIMENTAL DATA

VITAMIN B IN SUGARCANE JUICE

The juice used for these experiments was obtained from sugarcane of the 1926 crop, shipped in cold storage from Florida. As soon as possible after arrival the canes were crushed in a laboratory-size sugarcane mill, and the juice obtained was filtered through two thicknesses of cheesecloth. The fairly clear juice was then distributed into a large number of small wide-mouth bottles and placed in cold storage at a temperature below freezing. The frozen juice was taken out of one bottle at a time as needed and was allowed to liquefy at room temperature. The quantities required daily for the feeding experiments were measured out by means of a pipette.

A sample of the freshly expressed juice was analyzed in the carbohydrate division of this bureau. The results are as follows:

Brix, corrected to 20° C.....	degrees.....	14.64
Direct polarization, 20° C.....	do.....	9.73
True sucrose (invertase method).....	per cent.....	9.45
Solids (by drying).....	do.....	13.17
Invert sugar (Meissl and Hiller method).....	do.....	4.13
Apparent purity, $9.73 \times 100 \div 14.64$	do.....	66.46
True purity, $9.45 \times 100 \div 13.17$	do.....	71.76

The juice was tested for vitamin B by both the curative and the prophylactic methods. In the curative tests young albino rats were fed a vitamin B free ration consisting of casein, 20 parts; Osborn and Mendel's Salt Mixture IV, 5 parts; butterfat, 15 parts; and cornstarch, 60 parts. When evidence of vitamin B deficiency was manifested the cane juice was fed at definite levels daily apart from the basal ration. The rats were kept in individual cages having raised screen bottoms. The prophylactic tests were similarly conducted except that the cane juice was fed from the start. The results are shown graphically in Figure 1. Lot 1 consisted of two rats used for controls. Each curve of the other four lots represents the average weight of four rats fed the quantities of juice indicated on the chart. The rats of lots 2 and 3 were given the juice from the beginning of the experiment. In the curative tests with rats of lots 4 and 5, the period during which the juice was fed is indicated in each curve by the broken line.

On account of the difficulty of getting the animals of lot 2 to take as much as 20 c. c. of juice daily, the juice was concentrated to one-fourth of its original volume by evaporation at low temperature under diminished pressure. Of this product 5 c. c. was fed daily. From the results shown it appears that 15 c. c. of the cane juice fed daily fell short of supplying enough vitamin B to maintain weight, but that 20 c. c. was sufficient to promote growth at a fair rate for at least 60 days. Some of the rats of each lot did not always take all the juice given them, but it is believed that this did not significantly affect the final average results.

A previous shipment of cane of the 1924 crop had been received from Louisiana. This cane was superior in size, appearance, and in content of juice to that obtained from Florida. On account of unavoidable circumstances it was not possible to study the vitamins in the juice of this cane to the extent that had been planned. However, the curve in Figure 2, which represents the average weight of four

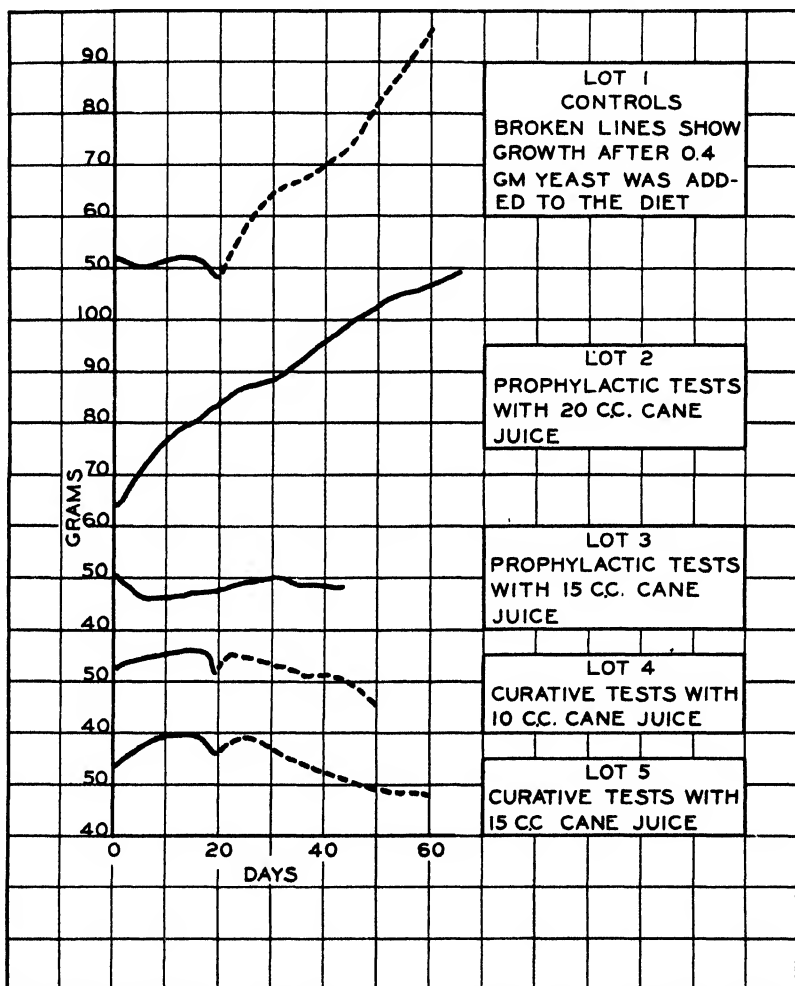


FIGURE 1.—Growth of rats when fed prophylactic and curative doses of cane juice as sources of vitamin B as compared with that of rats fed yeast

rats, shows that the juice from this lot of cane contained more vitamin B than did that of the cane obtained from Florida. Ten cubic centimeters daily of the juice was sufficient to enable the rats to resume growth with an average gain in weight of 15 gm. in 35 days, as indicated by the broken line in the curve. The composition of the basal diet was as follows: Meat residue, 18 gm.; Salts IV, 5 gm.; butterfat, 15 gm.; cornstarch, 62 gm.; making a total of 100 gm.

VITAMIN D IN SUGARCANE JUICE

Tests for vitamin D were made on the juice from the 1926 Florida sugarcane (previously referred to) by means of the "line test".⁶ One lot of four rats was brought to a rachitic condition by feeding them for 25 days Steenbock's rachitic ration No. 2965, consisting of yellow corn, 76 parts; wheat gluten, 20 parts, calcium carbonate, 3 parts; and sodium chloride, 1 part. The animals were then given daily 10 c. c. of the cane juice for 15 days. At the end of the period the degree of calcification induced in the radii of the rachitic animals was observed. A trace of calcification of the rachitic metaphyses was noted in the case of one rat. The others gave negative results.

VITAMIN A IN SUGARCANE JUICE

Preliminary tests for vitamin A indicated the presence of but little of this vitamin. Consequently, these studies were not continued further.

VITAMIN B IN SUGARCANE JUICE EXPRESSED AT DIFFERENT PRESSURES

The juice used in the following experiments was obtained from sugarcane of the 1927 crop sent from Franklin, La. Before shipment

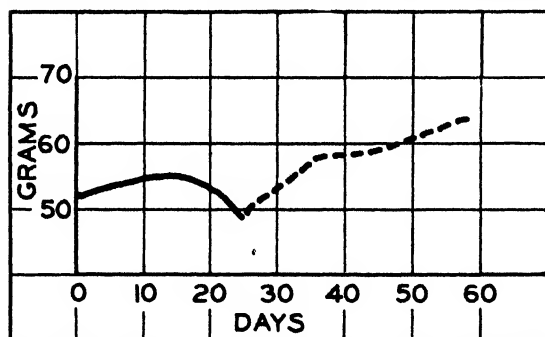


FIGURE 2.—Growth of rats when fed Louisiana cane juice of the 1924 crop as a source of vitamin B

the ends of the stalks were paraffined partially to protect them from deterioration. The shipment included 100 pounds each of stalks representing the upper third and lower third portions of the cane and 200 pounds of whole cane stalks.

Preliminary to the feeding experiments described later it was desired to ascertain the behavior of the experimental rats on the basal ration used when different levels of yeast furnished vitamin B. The results obtained may also serve as a basis of reference by which the responses of the animals to the sugarcane products tested in the experiments hereafter described can be compared. The vitamin B free basal ration consisted of casein, 18 parts; Osborne and Mendel's Salt Mixture IV, 4 parts; agar, 2 parts; cod-liver oil, 2 parts; and dextrin, 74 parts.

Figure 3 shows the growth response of the animals to different levels of yeast fed as a source of vitamin B. Each curve represents the average weight of four male rats. Although 0.4 gm. of yeast per day permitted growth at a rapid rate, an increased amount of yeast produced better growth. The quantity fed daily is indicated on the chart. On the basal diet used control rats receiving no vitamin B usually

⁶ MCCOLLUM, F. V., SIMMONDS, N., SHIPLEY, P. G., and PARK, E. A. STUDIES ON EXPERIMENTAL RICKETS. A DELICATE BIOLOGICAL TEST FOR CALCIUM-DEPOSITING SUBSTANCES. *Jour. Biol. Chem.* 51: 41-49, illus.

cease to gain in weight about the tenth day, and almost invariably lose weight rapidly after the fifteenth day.

Ten kilograms of the whole cane stalks were put through a laboratory-size sugarcane mill. The bagasse (5,150 gm.) was cut rather fine in a feed cutter, and pressed in a large Bushnell press, without use of maceration water, until all the juice that could be expressed was removed. The residual bagasse was then further pressed in a hydraulic press up to a pressure of 2,400 pounds per square inch. A total extraction of 70.6 per cent was obtained, of which 4.8 per cent was removed by the hydraulic press. As it was desired to compare only the juice expressed in the roller mill with that obtained from the hydraulic press, the intermediate fraction removed by the Bushnell press was discarded. The two fractions of the juice were filtered through two thicknesses of cheesecloth, and concentrated by evaporation under reduced pressure to one-fourth of the original volume. The concentrated product was stored at low temperature. The results of analyses of the high and low pressure juices are presented in Table 1.

As shown in Figure 4, the vitamin B content of the juice obtained at the higher pressure was much greater than that of any other sugarcane juice tested. Each curve represents the average weight of four male rats fed daily, apart from the basal ration, quantities of the concentrated product corresponding to the number of cubic centimeters of the original juice indicated at the right of the chart. The curves of lots 14 and 15 show that the high-pressure juice is about twice as potent in vitamin B as the other juice. A comparison of the curves in this chart with those in Figure 3 shows that 10 c. c. of the high-pressure juice was approximately equal to 0.15 gm. of dried yeast as a source of vitamin B.

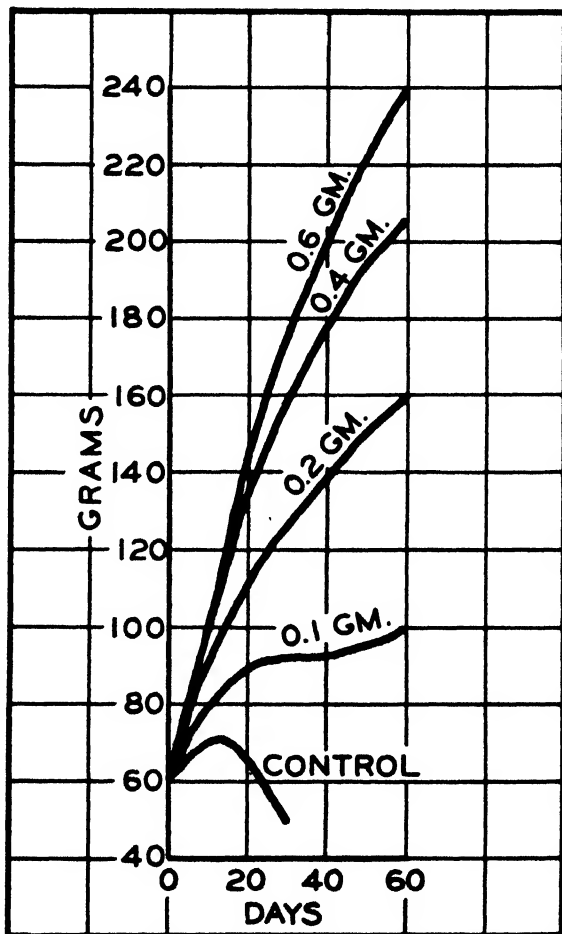


FIGURE 3 - Growth response of rats to different levels of yeast fed as a sole source of vitamin B

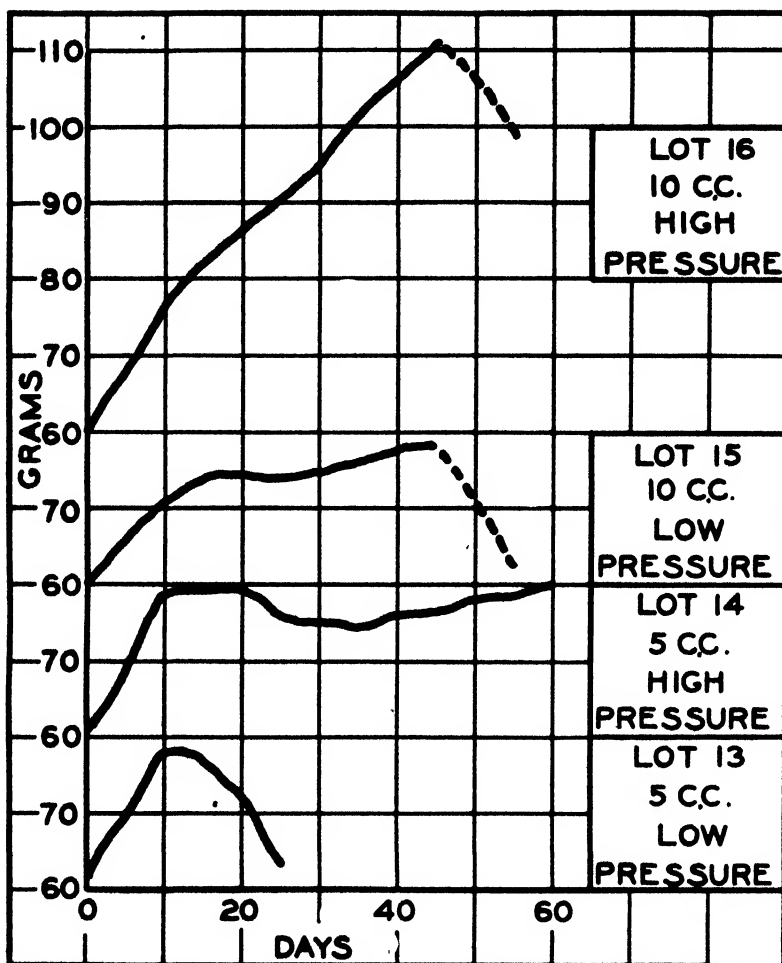


FIGURE 4.—Growth of rats when fed two levels of cane juice expressed at different pressures as a source of vitamin B

TABLE 1.—Analyses of cane juice expressed at high and low pressure

Item	Cane juice	
	High pressure	Laboratory cane mill
Brix.....	18.60	19.40
Apparent purity.....	71.23	80.89
Direct polarization.....	13.40	15.71
Invert polarization.....	-3.92	-4.77
True sucrose.....	13.95	15.60
Solids (by drying).....	17.83	19.01
True purity.....	78.24	82.06
Reducing sugars as invert.....	2.40	1.66

Two rats in lot 13 developed severe polyneuritis at 31 and 38 days, respectively. This symptom of vitamin B deficiency, however, was not observed in the other groups. Further evidence that growth in these experiments was not limited primarily by a lack of the pellagra-preventive factor is presented in the curves of lots 15 and 16. At the end of 45 days when the supply of high-pressure juice was exhausted, these lots received 5 per cent of autoclaved yeast. If the diet of these rats had contained an adequate supply of vitamin B, and growth had been limited by the pellagra-preventive factor, growth at the same rate as that shown for the first 10 days of the experimental period would be anticipated when the autoclaved yeast was added to the diet.

VITAMIN B IN SUGARCANE JUICE FROM THE UPPER AND LOWER THIRDS OF THE CANE STALKS

The upper and lower third portions of the cane stalks (100 pounds each) were run through the laboratory cane mill, and the juice was filtered through two layers of cheesecloth in order to remove finely divided particles of stalk. The top portions yielded 11,840 c. c. of juice, and the lower portions 16,880 c. c.

The juice was concentrated and stored as already described. Analysis of the juice gave the results shown in Table 2.

TABLE 2.—Analyses of juice obtained from the upper and lower third portions of sugarcane

Item		Juice from upper third	Juice from lower third
Brix	degrees	16.5	19.9
Apparent purity	per cent	81.0	90.0
Reducing sugars as invert sugar	do	1.4	0.5
True sucrose	do	14.04	18.13
Solids (by drying)	do	16.73	19.58
True purity	do	83.92	92.60
Direct polarization	degrees	+13.55	+17.93
Invert polarization	do	-4.91	-5.91

As shown in Figure 5, the upper part of sugarcane stalks contains more vitamin B than the lower part of the same stalks. The animals of lots 26 and 28 received juice from the lower third of the stalks, and lots 27 and 29 received juice from the upper third. Each curve represents the average weight of four male rats fed the daily dose indicated at the right of the chart. One rat in lot 26 and two rats in each of lots 27 and 28 failed to survive the experimental period of 60 days. Seven cases of polyneuritis were observed, as follows: In lot 26, two at 38 and 60 days; in lot 27, two at 45 and 48 days; in lot 28, two at 53 days, and one at 56 days.

VITAMIN B IN SUGARCANE CREAM

Sugarcane cream is a new product developed by the carbohydrate division of this bureau.⁷ It is prepared by evaporating cane sirup in an open kettle to a high predetermined density, judged by its boiling temperature; cooling this sirup and crystallizing a portion of

⁷ PAINE, H. S., and BALCH, R. T. SUGAR-CANE CREAM A NEW PRODUCT OF COMMERCIAL VALUE. U. S. Dept. Agr. Yearbook 1927. 605-607. 1928.

the sugar therein with continuous stirring with a "cream beater" similar to that used in making sugar fondant for coated confectionery; and warming slightly the finished cream in a jacketed remelt kettle, in order that the cream may flow into the containers of tin or glass.

Examination of this product for vitamin B was conducted as in the preceding experiments. The cane cream was fed daily apart from the basal diet at the levels indicated in Figure 6. Each curve represents the average weight of four rats. The results show that cane

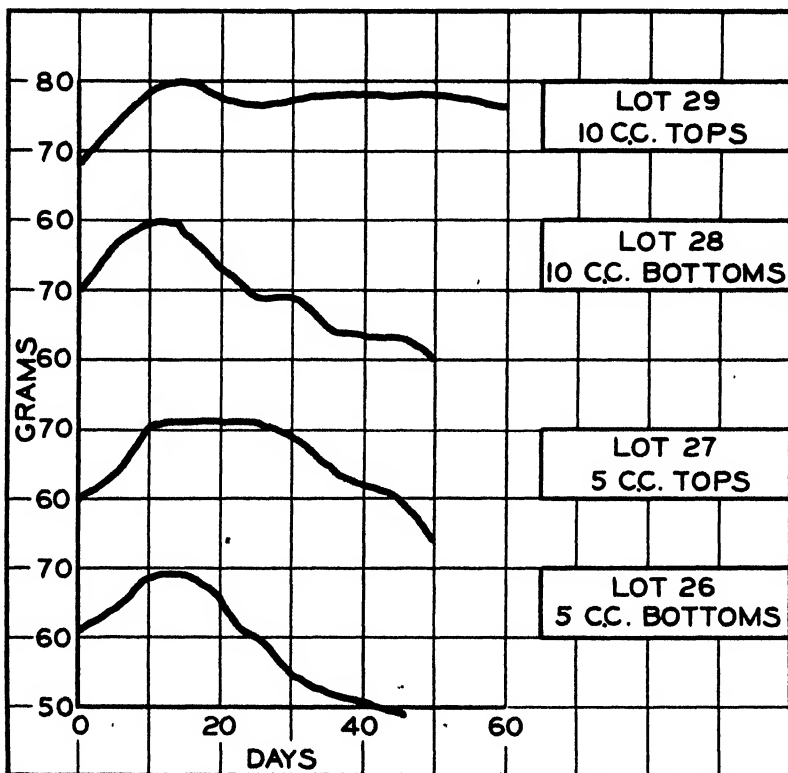


FIGURE 5. Growth of rats when fed cane juice obtained from different portions of the stalk, as a source of vitamin B

cream contains no vitamin B. The average survival of four male rats in lot 6 was 34½ days; in lot 7, 32 days; and in lot 8, 34 days. Three cases of polyneuritis occurred in lot 6, three in lot 7, and one in lot 8.

VITAMIN B IN SUGARCANE SIRUP AND BLACKSTRAP MOLASSES

Sugarcane sirup and blackstrap molasses were also obtained from the carbohydrate division. The cane sirup had been prepared from sugarcane juice by the Georgia open-pan process. Both Louisiana and Porto Rico blackstrap molasses were examined. Instead of being fed apart from the basal ration as in all the preceding experiments, these products were mixed with dextrin and incorporated in the

ration. The ration was the same as that previously used except that the sirup and molasses replaced an equal weight of dextrin. The average daily intake per animal of the products tested is shown in Table 3, and the growth of the rats fed these products is shown in Figure 7. Each curve in this figure represents the average weight of four male rats. That the products tested did not contain significant amounts of vitamin B is apparent from the fact that no appreciable prolongation of life resulted from their addition to the diet. No case of polyneuritis was observed in lot 56, but in each of the other lots two cases were noted.

TABLE 3.—Average daily intake per animal of cane products tested

Item	First week	Second week	Third week	Fourth week
	Gram	Gram	Gram	Gram
Cane sirup.....	0.41	0.43	0.33	0.24
Porto Rican molasses, 10 per cent.....	.97	.96	.63	.37
Porto Rican molasses, 5 per cent.....	.48	.56	.33	.28
Louisiana blackstrap.....	.54	.53	.20	.26

DISCUSSION

Probably the most important practical application of these findings is concerned with the use of cane molasses as a food for both human and animal consumption. During the last 15 or 20 years immense quantities of molasses have been used in the manufacture of so-called molasses feeds. From the results of the earlier work of Nelson, Heller, and Fulmer^b it is concluded that there are large variations in the vitamin B content of molasses, since the blackstrap molasses which they investigated was found to be an excellent source of this vitamin, whereas the writers found vitamin B to be absent from two samples of this product.

The results obtained in the present experiments show that the inclusion of generous amounts of molasses in the diet does not insure an adequate amount of vitamin B.

The fact that the upper portion of the cane stalk is richer in vitamin B than the lower portion may be associated with the difference in the

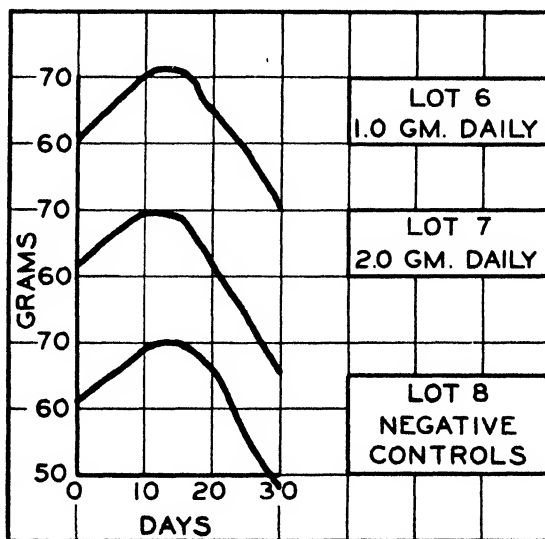


FIGURE 6.—Growth of rats when fed cane cream at different levels as a source of vitamin B

^b NELSON, V. E., HELLER, V. G., and FULMER, E. I. MOLASSES AS A SOURCE OF VITAMIN B. *Indus. and Engin. Chem.* 17: 199-201, illus. 1925.

stage of maturity of the different parts of the stalk. The older portion has become a storage organ while the younger portion is still a seat of considerable metabolic activity.⁶ While synthesis of sugar takes place in the leaves and these carbohydrates are then translocated to the stalk, changes in the nature of the sugar in the stalk continue until the stalk reaches maturity.

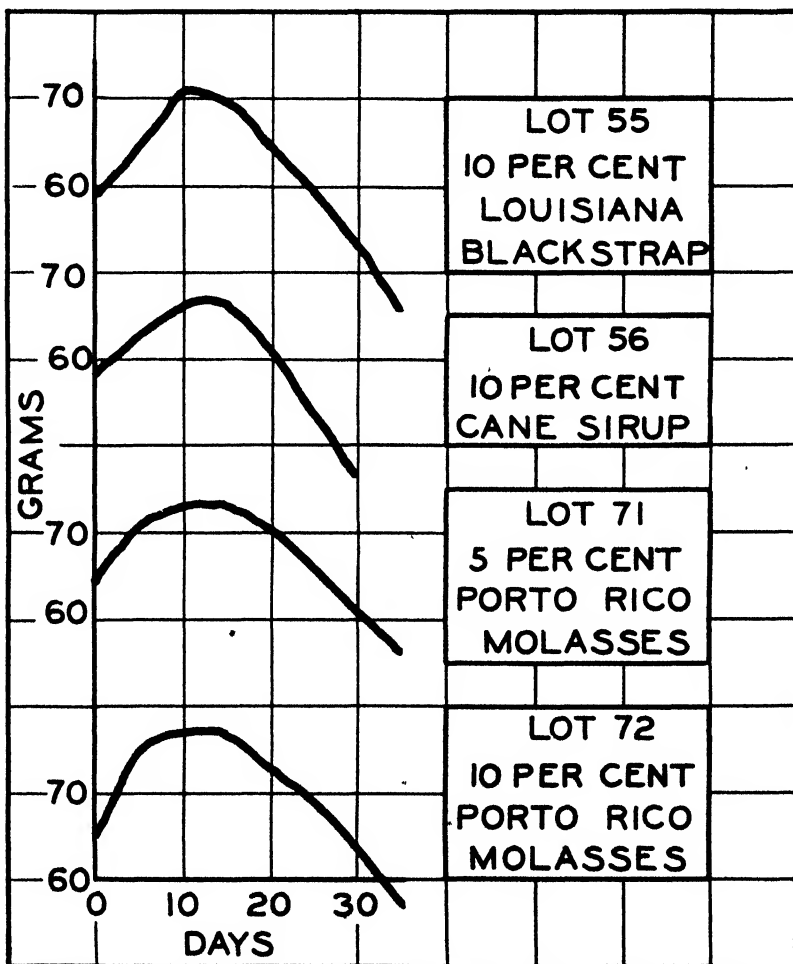


FIGURE 7.—Growth of rats when fed various cane-juice products as sources of vitamin B

Probably the contents of the thin-walled parenchymal cells which contain the sugar are most easily expressed so that the ordinary sugarcane juice does not contain a large portion of other protoplasmic material. By applying much higher pressure the protoplasm of other cells was expressed. The sugar is reserve food for the young plants which develop from the nodes when the stalk comes in contact

⁶ DEER, N. CANE SUGAR: A TEXTBOOK ON THE AGRICULTURE OF THE SUGAR CANE; THE MANUFACTURE OF CANE SUGAR, AND THE ANALYSIS OF SUGAR-HOUSE PRODUCTS. Ed. 2, rev. and enl., 644 p., illus. London. 1921.

with the ground or is buried in it. It seems that there is more vitamin B in those cells in which there is the greatest metabolic activity.

SUMMARY

The results of studies of the vitamin content of sugarcane juice and some commercial products made from it are reported.

Sugarcane juice is a poor source of the antineuritic vitamin. The juice from the upper portions of cane stalks is richer in this vitamin than juice from the lower portions. Juice obtained from bagasse by using high pressure is richer in the antineuritic vitamin than ordinary cane juice.

Sugarcane juice contains a small amount of vitamin A and little, if any, vitamin D.

Cane sirup, Louisiana and Porto Rico blackstrap molasses, and cane cream, products made from sugarcane juice, were found to be devoid of demonstrable quantities of vitamin B.

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No. 11

CONIDIAL FRUCTIFICATIONS IN *BALANSIA* AND *DOTHICHLOE*¹

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INTRODUCTION

Balansia and *Dothichloe* are generally recognized as parasites upon grasses and sedges, although the conidial fructifications of these fungi have usually escaped observation because they are ephemeral and inconspicuous. These two genera have been distinguished chiefly by the form and position of the ascogenous fructification. The stroma of *Balansia* is defined as capitate and stipitate and that of *Dothichloe* as effuse. *Dothichloe* by definition has an effuse ascogenous stroma resembling *Epichloe* Tul. except that it is black. Certain species show intermediate characters in this respect, and there is little actual structural difference implied by these generic distinctions. Other structures such as conidial fructifications should be useful in clarifying the generic concepts, especially since so little has been known in regard to conidial fructifications in either genus. The importance of recognizing conidial stages is obvious, since their usually inconspicuous occurrence is overlooked even when correlated with floret sterility or abortion.

This paper presents certain observations on conidial phases thought to be significant in these genera. Cultural study of the organisms has been limited to moist-chamber development, since attempts at cultivation upon artificial media have thus far been ineffective. Ascospore germination followed by scattered conidial production, not in a fructification, as illustrated by Brefeld (4, pl. 5, p. 59-62)² for *Epichloe typhina* Tul. and by Moeller (10, pl. 5) for *Balansia*, are not discussed here because they are not regarded as especially pertinent. All specimens referred to have been deposited with the mycological collections of the Bureau of Plant Industry.

BALANSIA

BALANSIA CLAVICEPS AND EPHELIS MEXICANA

Balansia claviceps Speg. (11, p. 45-46), the type species of the genus, was described as having the capitate ascogenous stroma borne upon a sclerotium formed upon an unknown grass in Bolivia. No conidial fructifications were mentioned, nor has any conidial stage since been recorded for it.

Balansia trinitensis Cke. and Mass. (5) is a very similar species, described, however, with illustrations of a conidial fructification assigned to the form genus *Ephelis* as *E. trinitensis* Cke. and Mass. The conidia are said to be borne upon short, simple conidiophores in a characteristic discoid fructification.

¹ Received for publication July 23, 1930; issued December, 1930.

² Reference is made by number (italic) to Literature Cited, p. 766.

The form genus *Ephelis* Fr. (9, p. 370) is based upon *E. mexicana* Fr. sec. B. and C. (3, p. 353). This species grows upon various grasses in Mexico, Florida, and the West Indies. (Pl. 1.) Living

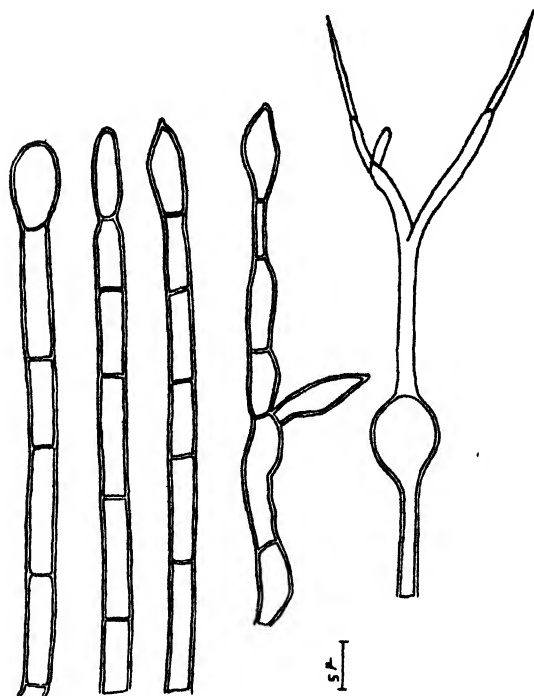


FIGURE 1.—Exelipular hairs of *Ephelis mexicana*, aberrant branched hairs at the right, on *Cenchrus* from Florida

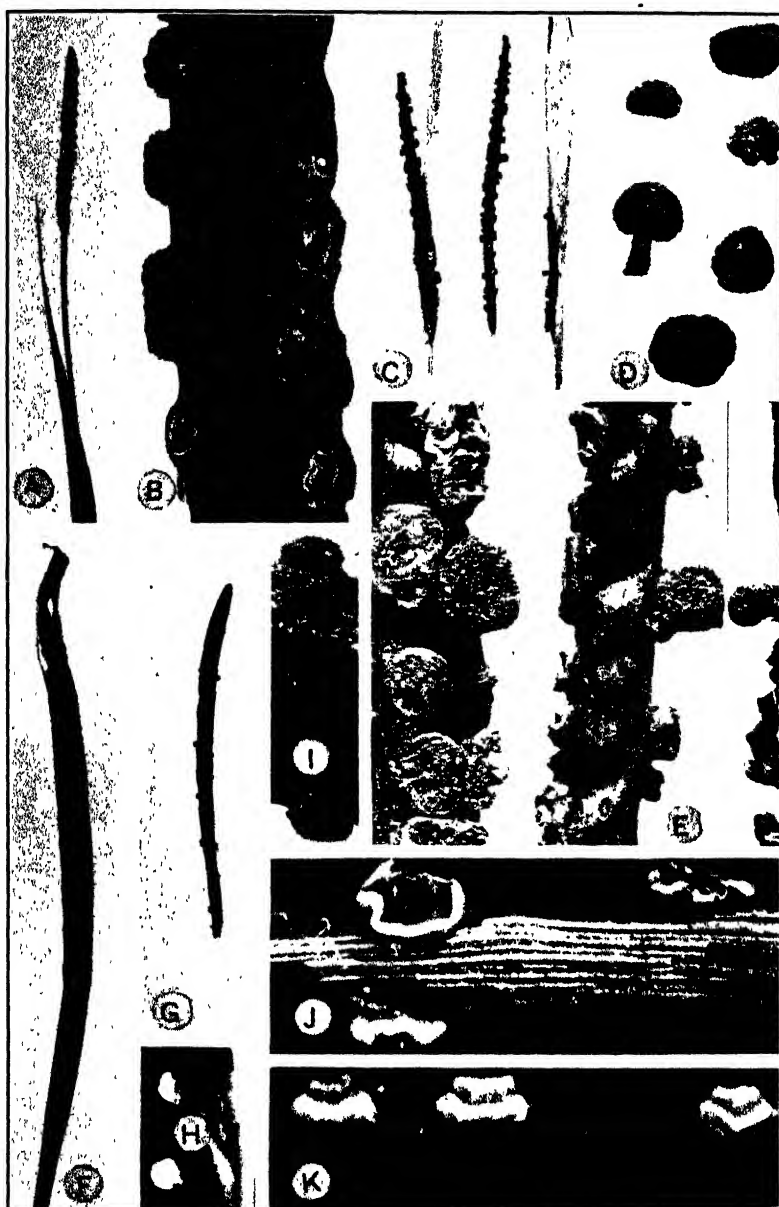
material of *E. mexicana* growing on *Cenchrus* in Florida has been available for study at different times for several years through the kindness of G. F. Weber, A. J. Bruman, A. C. Foster, H. R. Fulton, and G. B. Sartoris. The *Ephelis* fructification develops from certain points beneath the black, laccate rind of the sclerotium as a globular, hairy protrusion which opens, becoming discoid. (Pl. 1, H-K.) The rim and lower surface of the disk are an exciple covered with long hairs (fig. 1)³ 3 μ to 4 μ in diameter. The upper surface of the disk is a grayish layer of needle-shaped conidia borne as a palisade upon conidiophores at first simple, then verticillately branched. (Fig. 2.) Under certain conditions the conidia ex-

trude in conical to columnar masses. When mature the fructifications are usually but not always discoid, simulating apothecia of an ascomycete. This similarity induced Fries (9) to describe the fungus as a discomycete. This type of fructification is usually regarded as a

EXPLANATORY LEGEND FOR PLATE 1

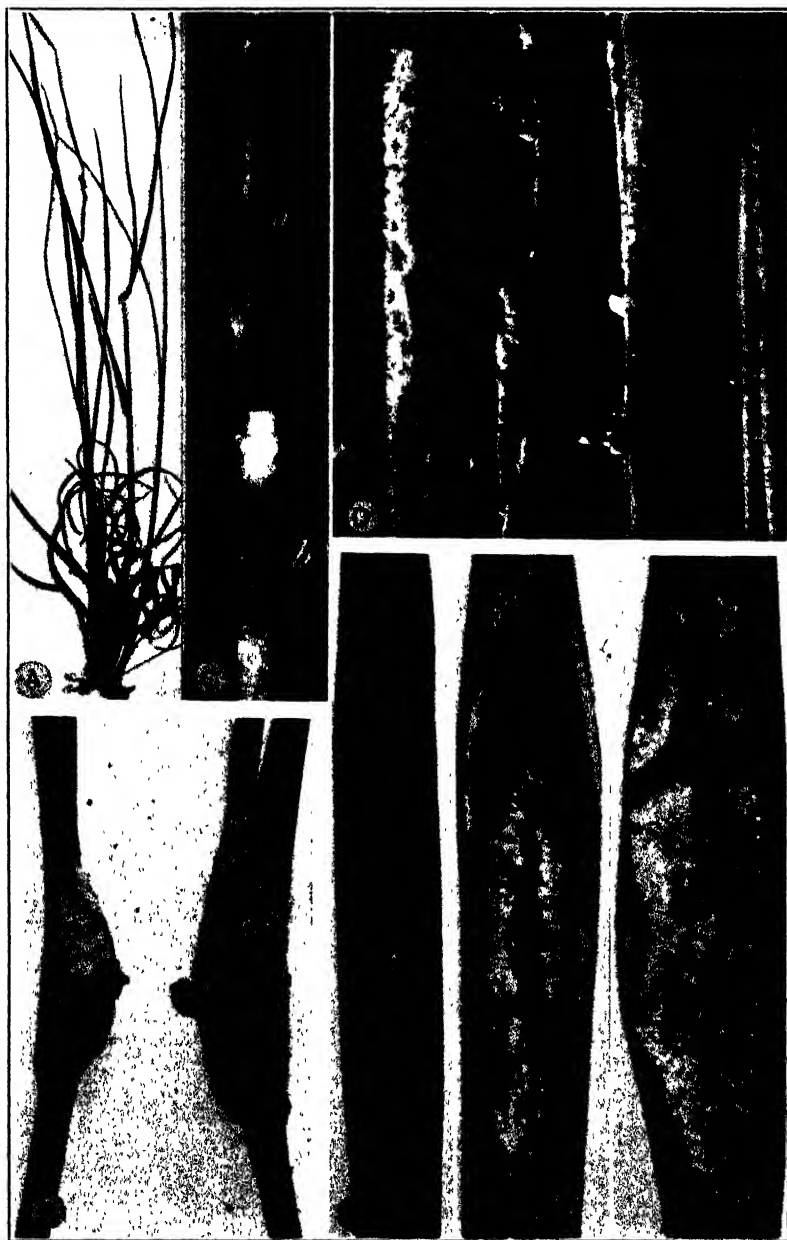
- A.—*Ephelis mexicana* on *Chaetochloa geniculata*; Central Hershey, Cuba, February 3, 1924, J. A. Stevenson; sclerotium covering the aborted inflorescence, and bearing typical fructifications. $\times 1$.
 B.—Detail of A. $\times 12$.
 C.—*Ephelis mexicana* on *Chaetochloa* sp.; Santiago de las Vegas, Cuba, August 29, 1907, H. Hasselbring, sclerotium bearing *Ephelis* and ascogenous (*Balanista* sp.) fructifications. $\times 1$.
 D.—Data of C.; detached asexual stromata. $\times 8$.
 E.—Detail of C. $\times 8$.
 F.—*Ephelis mexicana* on *Cenchrus echinatus*; Lake City, Fla., January 28, 1894, P. H. Rolfs, sclerotium covering the aborted inflorescence, partly inclosed by leaf sheath. $\times 1$.
 G.—*Ephelis mexicana* from *Cenchrus echinatus*; Gainesville, Fla., March 27, 1923, G. F. Weber, detached sclerotium bearing *Ephelis* fructifications. $\times 1$.
 H.—*Ephelis mexicana* on *Cenchrus echinatus*; Pierson, Fla., December 20, 1927; initial appearance of *Ephelis* fructifications on sclerotia developed in the moist chamber. $\times 12$.
 I.—Data of H; partially developed *Ephelis* fructifications after 24 hours in a moist chamber. $\times 12$.
 J.—Data of H; mature *Ephelis* fructifications. $\times 12$.
 K.—Data of I; mature *Ephelis* fructifications with adherent spore mass, spores here produced chiefly upon verticillately branched conidiophores. $\times 12$.
 Photographs for C, D, and E by J. F. Brewer; the others by M. L. S. Foubert.

³ Drawings for figs. 1, 2, and 3 were made from living material mounted in water.



EPHELIS MEXICANA

FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



BALANSIA AND DOTHICHLOE

FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

pycnidium and assigned to the Excipulaceae, although its structure, disregarding the peculiar exciple, is suggestive of a sporodochium.

A noteworthy specimen of *Ephelis mexicana* on *Chaetochloa* gathered in Cuba in 1907 by H. Hasselbring is available through his kindness. It bears a few ascogenous stromata of *Balansia*. (Pl. 1, C and D.) This is most likely congeneric with a specimen of *Balansia clariceps* Speg. presented to the writer by the late Professor Spegazzini, but appears to be specifically distinct.

BALANSIA HYPOXYLON

Balansia hypoxylon (Pk.) Atk. (2) growing upon various grasses in the eastern half of the United States forms small whitish to grayish-green sclerotia surrounding the immature inflorescence, which it mummifies. (Pl. 2, A and B.)

The surface of the young sclerotium is made up of a palisade of swollen conidiophores, each with a single sterigmatalike apical projection bearing an ovate conidium. (Fig. 3, A.) These conidia were observed by Atkinson (2) in a single specimen sent to him from Texas, but he was doubtful of their significance. This conidial layer and conidia are somewhat similar to and probably homologous with the *Sphacelia* stage which was shown by Tulasne for *Epichloe* (12, p. 647) and for *Claviceps*

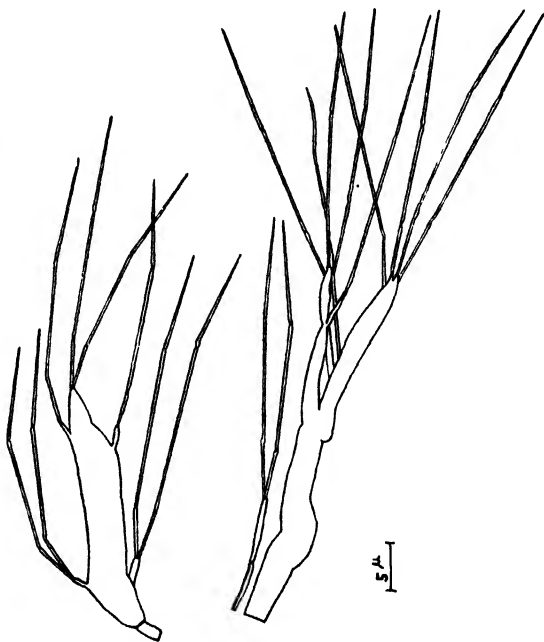


FIGURE 2—Conidia and conidiophores of *Ephelis mexicana* on *Cenchrus* from Florida

(11, 13). The conidiophores of *Claviceps* do not normally have apical projections or sterigmata as is the case in *Balansia hypoxylon*. In the closely related genus *Epichloe* there is, however, a sterigmatalike apical projection on the swollen conidiophore, generally ignored in

EXPLANATORY LEGEND FOR PLATE 2

A.—*Balansia hypoxylon* (diseased specimen of *Danthonia spicata*, Barcroft, Va., June 2, 1929, H. A. Allard), showing inflorescence covered by fungous tissue or sclerotium. $\times 1$.

B.—*Balansia hypoxylon*, data of A, sclerotia upon aborted inflorescence covered by the grayish amerosporous fructification, each sclerotium bearing an *Ephelis* fructification. $\times 8$.

C.—*Dothichloe aristidae* on *Panicum* sp.; Arlington Experiment Farm, Rosslyn, Va., June 20, 1929, W. W. Diehl: a, Effuse, grayish scolecosporous layer covering culm and aborted inflorescence ($\times 12$); b, remnant of scolecosporous fructification with immature ascogenous stroma ($\times 9$).

D.—*Dothichloe atramentosa* on *Sporobolus berterianus* (Auburn, Ala., July 17, 1929, J. L. Seal), showing masses of scoleo spores extruding from between folds of the leaves. $\times 14$.

E.—*Dothichloe atramentosa*, data of D; amerosporous layer developed upon immature ascogenous stroma after exposure for 48 hours in a moist chamber. $\times 14$.

Photographs for A and B by J. F. Brewer; others by M. L. S. Foubert.

illustrations but correctly shown in Delacroix's Atlas (6, pl. 48, fig. 29). These a-merosporous fructifications of *B. hypoxylon* and of *Epichloe typhina* (*Sphacelia typhina* Sacc.) are practically identical, suggesting a close relationship. After the abscission of the conidia in *B. hypoxylon* the sclerotium remains gray to gray green in color, due to the covering of conidiophores. The sclerotium is at first gelatinous but becomes cartilaginous in texture; it never forms a laccate rind like that covering the sclerotium of *E. mexicana*.

These sclerotia later bear a discoid *Ephelis* stage (pl. 2, B), described originally from a Canadian specimen as *Ephelis borealis* E. and E. (8, p. 86). The fructification, scolecospores, and conidiophores are very similar to those of *E. mexicana*, but differ in that in the northern species the excipular hairs are interspersed with the peculiar ovate

conidia. It appears that excipular hairs of *Balansia hypoxylon* may be homologous with elongated conidiophores which remain sterile, since hairlike sterile structures may be produced in mass upon the surface of the sclerotium when in contact with water. (Fig. 3, A.)

These observations on *Balansia hypoxylon* are based upon living material growing upon *Danthonia spicata* obtained chiefly at Barcroft,⁴ Arlington Co., Va., in 1929, as

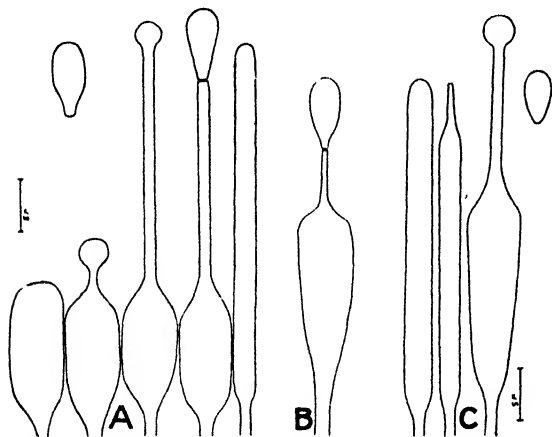


FIGURE 3 - A-merosporous conidia and conidiophores. A, *Balansia hypoxylon* (an aberrant conidiophore at the right) on *Danthonia* from Virginia; B, *Dothichloe atramentosa* on *Sporobolus* from Alabama; C, *D. aristidae* on *Panicum* from Virginia

well as upon various dried herbarium specimens. At Barcroft both types of fructifications were first observed early in June. By July and August of 1929 the formation of ascogenous stromata had apparently completed the activities of the fungus for the season.

BALANSIA CYPERI

Balansia cyperi Edg. (7) has acicular, *Ephelis*-like conidia in pink masses on indefinite areas of the surface of the white sclerotia. These conidial areas lack the definite excipular structure of *Ephelis*. The species has not been available to the present writer in a living condition, and hence it is not advantageous to discuss its relations further here.

DOTHICHLOE

The genus *Dothichloe* Atk. (1, p. 223), with *D. atramentosa* (B. and C.) Atk. (2, p. 260-264) as the type species, was distinguished because of its black, effuse ascogenous stroma. This species on grass blades appears to be common in the southeastern part of the United

⁴The writer is grateful to H. A. Allard for reporting this colony of diseased plants.

States and in the West Indies. In living material on *Sporobolus berterianus* from Auburn, Ala., generously contributed in 1929 by J. L. Seal, it was observed that preceding the development of the ascogenous stroma and coincident with the unrolling of the young grass blade there is formed an effuse palisade layer of acicular conidia (scolecospores). From beneath the folds of the grass blade the conidia extrude in waxy masses. (Pl. 2, D.) Similar material in an early stage of development growing on an unknown grass at Burgaw, N. C., was observed by the writer on April 25, 1930. The conidia and conidiophores are practically identical with those of *Ephelis mexicana* and of the *Ephelis* stage of *Balansia hypoxylon*. This effuse conidial layer is, however, not circumscribed by any definite exciple. The examination of herbarium material of the species has disclosed the presence of the scolecosporous fructifications in specimens from various localities. This scolecospore stage is later displaced by the effuse ascogenous stroma. When the stroma, as it approaches maturity, is placed in a humid atmosphere for 12 to 48 hours it develops upon its surface a whitish to grayish-green amerosporous layer (pl. 2, D, and fig. 3, B) resembling that in *B. hypoxylon*.

A closely related species is *Dothichloe aristidae* Atk. (1, p. 224). It surrounds the culms of various grasses, appearing as a black equivalent of *Epichloe typhina*, and is broadly distributed in North and South America. Living material growing upon *Panicum* sp. at the Arlington Experiment Farm, near Rosslyn, Va., in 1929, showed a scolecosporous fructification (pl. 2, C) followed by the ascogenous stroma which under certain moisture conditions would produce an amerosporous stage. (Fig. 3, C.) The conidial fructifications of *D. aristidae* are therefore practically indistinguishable microscopically from those of *D. atramentosa* and develop in the same sequence.

SUMMARY

In *Balansia hypoxylon* and in the known species of *Dothichloe*, fungus parasites of certain grasses, conidial fructifications hitherto unknown or rarely recognized are found to develop in a similar manner.

An amerosporous conidial fructification similar to that of *Epichloe* is reported for two species of *Dothichloe* and for *Balansia hypoxylon*.

An ascogenous fructification referable to *Balansia* is reported for *Ephelis mexicana*, for which no amerosporous stage has been found.

An effuse conidial fructification microscopically similar to the *Ephelis* (scolecosporous) stage of *Balansia* is reported for *Dothichloe*.

Branched conidiophores are reported in the *Ephelis* fructifications of *Balansia hypoxylon*, in *Ephelis mexicana*, and in the scolecosporous fructifications of *Dothichloe*.

The character and sequence of the conidial fructifications in *Balansia* and in *Dothichloe* show definite correlations which suggest their recognition in the taxonomic interpretation of these fungi.

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FURTHER STUDIES ON THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSICOCHEMICAL PROPERTIES OF THE LEAF-TISSUE FLUIDS OF COTTON¹

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INTRODUCTION

While the problem of the relationship between the chemical and physical properties of the soil and the characteristics of the plant is as old as the beginnings of scientific agriculture, it is only recently that the investigation of certain phases of the problem by means of the determination of correlations between the properties of the soil and the characteristics of crop plants as grown in the experimental field has been undertaken.

In the many studies that have been made of the influence of the nutrient solution on the characteristics of the plant, the method almost universally employed has been that of culture in synthetic solutions of known composition. Unquestionably this technic has yielded results of great importance. It is also quite clear that this method must furnish the final criterion for the determination of the rôle of various elements in the metabolism of the plant.

There are, however, many difficulties connected with investigations involving such methods. Plants are grown under conditions very different from those that are normal for their development. Notwithstanding the many efforts that have been made to eliminate the influence of these abnormalities of condition on the development of the organism, it is quite clear that plants grown in culture solutions do not develop in exactly the same way as those grown in the field. It therefore seems desirable to supplement water-culture and pot-culture methods by others whenever possible. One such accessory method is to consider the relationship between soil properties in the field and the characteristics of the plants produced. While field experiments can not be expected to give results of the same order of finality as water-culture and pot-culture methods in the determination of the relative importance of individual constituents of the soil solution in the metabolism of the plant, they nevertheless have two advantages:

(1) The investigations are conducted on plants growing under natural conditions. Any results secured will, therefore, be such as may be expected in the actual growth of crop plants in the field.

(2) By the proper development of agronomic, chemical, and biometric technic, the investigations may be carried out simultaneously

¹ Received for publication June 13, 1930; issued December, 1930. The term "soil solution" is preferred to "soil extract" in this paper since the argument throughout is directed toward the content of chlorides and sulphates which are present in the soil as the environment of root growth.

² Died Apr. 24, 1930.

with others on crop plants as grown under conditions of agriculture, or at least of large-scale field experimentation. This combination of agronomic and physiological technic has obvious practical advantages.

The purpose of the present paper is to report further investigations on the relationship between the concentration of the soil solution and the tissue-fluid properties of the Egyptian and upland types of cotton.

The first study (6)³ dealt with the relationship between the electrical resistance of the soil on the one hand and the osmotic concentration (as expressed in terms of freezing-point depression), specific electrical conductivity, and chloride content of the leaf-tissue fluids of the plant on the other.

In a subsequent paper (8) evidence was adduced to show that under certain conditions there is a positive correlation between soil salinity and seedling stand in cotton. Finally it seemed desirable to extend investigations to the consideration of the relationship between the properties of the soil and some functional characteristics of the plant. An analysis (9) of records of Pima Egyptian, Meade upland, and Acala upland cotton indicates that the flowering date is slightly retarded by soil salinity.

In the earlier investigation (6) the electrical resistance of the saturated soil mass, as determined by the electrical bridge in the form described by Davis and Bryan (4), provided the only available measure of the concentration of the soil solution. Although this method was not regarded as altogether satisfactory, it seemed desirable to employ it in a preliminary investigation, rather than the much more expensive analytical methods. While the soil bridge has been extensively used in soil surveys and in other investigations, it is quite evident from the theory involved that it can not give information concerning the identities of the solutes to which the conductance of the saturated soil mass is due. This limitation has always been recognized. It is, therefore, desirable that actual analytical determinations be made of the solutes of the soil to which its electrical conductivity is due. In this paper the studies of soil salinity are extended to include analytically determined chloride and sulphate contents.

The determination of these ions is particularly desirable in any consideration of the relationship between the salinity of the soil and the characteristics of cotton. Not only is the cotton plant capable of high salt tolerance, as indicated by general agricultural experience, as suggested by observations made by Balls (2, 3), and as established in early studies by Kearney and Means (15) and by Kearney (14), but the salt relations of the tissue fluids have been shown to be of peculiar interest. Investigations made for the Bureau of Plant Industry have shown not only that the chloride content (12) and the sulphate content, (10) of the leaf-tissue fluids are high, but also that there is differential absorption of these anions (5, 7, 11, 13). Furthermore, the chloride content of the tissue fluids, especially in the Egyptian type of cotton (6), has already been shown to be highly correlated with the electrical resistance of the soil.

³ Reference is made by number (italic) to Literature Cited, p. 788.

For these reasons it seemed particularly desirable that an investigation be made of the relationship between the concentration of the chloride and sulphate ions in the soil on the one hand and the physicochemical properties, especially the chloride and sulphate contents of the leaf-tissue fluids, on the other.

METHODS

DETERMINATION OF SOIL CONSTITUENTS

The electrical resistance of the saturated soil mass was measured by the conventional method as indicated above.

The chloride and the sulphate content were determined on 30 to 50 gm. fractions of the air-dried, finely pulverized, and thoroughly mixed soil. These were transferred to 300 c. c. Florence flasks and 200 c. c. of a 5 per cent solution of ammonium nitrate (NH_4NO_3) was added to each flask. After 24 hours, during which time the suspensions were agitated frequently to produce a uniform suspension, a clear soil extract was obtained by filtering through quantitative filter paper. Through the use of the 5 per cent NH_4NO_3 solution as a solvent, the soil colloids were precipitated, and little difficulty was encountered in the subsequent filtrations. In the determination of chloride content, duplicate 25 c. c. portions of the filtrate were titrated against a tenth-normal solution of silver nitrate (AgNO_3), with 1 c. c. of a 5 per cent solution of potassium chromate (K_2CrO_4) as an end-point indicator (1, p. 87). In the determination of sulphate content, 100 c. c. portions of the filtered soil extract (obtained as above) were acidified with 5 c. c. concentrated hydrochloric acid (HCl) and heated to boiling on a hot plate. To the hot solution 10 c. c. of a 10 per cent solution of barium chloride (BaCl_2) was added with constant stirring, and the boiling was continued until the precipitated barium sulphate (BaSO_4) settled to the bottom of the beaker. The precipitate was collected on a quantitative filter paper, washed, dried, and ashed to constant weight in platinum.

DETERMINATION OF TISSUE-FLUID PROPERTIES

The methods employed are those which have been used in the various investigations on the tissue-fluid properties of cotton discussed in papers already published (5, 6, 10, 12, 13).

STATISTICAL ANALYSIS OF DATA

Correlation between soil properties and tissue-fluid properties have been determined by the usual product-moment method. Special methods of comparison will be indicated as required.

MATERIALS

The cultures considered are those of an experiment made in 1923. This set of cultures has furnished data on the chloride content (12) and the sulphate content (10) of the tissue fluids and on the relationship between soil salinity and seedling stand (8). The last-named paper (8) may be consulted for details of arrangement of plantings. In the consideration of the relationship between soil salinity and seedling stand (8) the area of the field used was limited to 16 rows, of 90 hills each, of the two varieties of cotton studied. (Hybrid

plants grown between the sections or plots of the two parental types were disregarded.) This course was followed because conditions at the north half of the plots were such that only a very few hills produced seedlings at all. To have included this area would have resulted in highly abnormal distributions of the number of seedlings per hill, since high modal frequencies on the zero class would have been obtained.

In the present investigation it has seemed desirable to include the determinations made on the few plants growing on the extremely saline north half of the experimental plots (D 1-10, D 1-11). This will be more readily understood if constants for the tissue fluids are first explained.

As noted in the papers on chloride content (12) and sulphate content (10), two series of determinations were made on the leaf-tissue fluids, the first based on collections taken from July 29 to August 14, and the second on samples taken from August 18 to 31.

The first series of determinations was made only on the plants on the south half of the plots, since those on the north half were so small that it was quite evident that samples of tissues for two sets of analyses would not be available. In consequence of this limitation, the correlations of the present paper are based in part on plants grown on the south half of the plot and in part on plants grown on both halves of the plot. Series 1 includes the correlations based on the first set of determinations of tissue-fluid properties. Series 2-B includes the correlations based on the second set of determinations but limited to the south half of the plot. It is therefore exactly comparable with series 1 as far as experimental area is concerned. Series 2-A includes all the tissue-fluid determinations based on plants growing on both the north and the south halves of the experimental plot.

RESULTS

All the correlation coefficients expressing the relationship between the measures of soil salinity and the properties of the leaf-tissue fluids are presented in Tables 1 to 3. The first column gives the variables of the soil (electrical resistance, R ; chloride content, Cl ; and sulphate content, SO_4) and of the plant (freezing-point depression, Δ ; specific electrical conductivity, κ ; chloride content, Cl ; and sulphate content, SO_4) involved in the correlations denoted by the symbols $'R\Delta$, $'R\kappa$, $'RCl$, $'RSO_4$, etc. The third to the tenth columns, inclusive, give the coefficients based on the determinations made on the soil layers of the first 4 feet. The eleventh and twelfth columns give the coefficients based on the average resistance of the first 4 feet. Finally, the thirteenth and fourteenth columns give the average of the coefficients based on the constants for the four individual soil layers.

As indicated above, two sets of determinations on the leaf-tissue fluids were available. Table 1 gives the coefficients measuring the relationship between the properties of the soil and the first series of determinations on the leaf-tissue fluids. Tables 2 and 3 give comparable results for the second series of determinations. Table 3 presents the constants for the south half of the field. It is therefore altogether comparable with Table 1. Table 2 gives the data from the second series of determinations for both parts of the experimental plot. These coefficients represent the fundamental constants upon which the subsequent discussion is based.

TABLE 1.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity (electrical resistance, chloride content, and sulphate content) and various tissue-fluid properties in Pima Egyptian and Long Star upland cotton grown at the United States Field Station, Sacoaton, Ariz., in 1923

[Experiment 1/23, series 1, determinations based on plants grown on south half of plot]

Variables	Correlation	Coefficient for—										Average correlation coefficient
		First foot *		Second foot *		Third foot *		Fourth foot *		All layers *		
		Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	
Resistance and—	$r R \Delta$	-0.3214 ± .0762	-0.1772 ± .0830	-0.4611 ± .0669	-0.2221 ± .0814	-0.4540 ± .0675	-0.3116 ± .0773	-0.4520 ± .0676	-0.2925 ± .0783	-0.5044 ± .0634	-0.3008 ± .0779	-0.4221
	$r R \kappa$	-0.2228 ± .0821	-0.1741 ± .0831	-0.5350 ± .0616	-0.1014 ± .0848	-0.4091 ± .0719	-0.0429 ± .0855	-0.3641 ± .0749	-0.0329 ± .0856	-0.4677 ± .0675	-0.0988 ± .0848	-0.3828
	$r R Cl$	-0.1203 ± .0638	-0.0330 ± .0849	-0.2789 ± .0784	+0.0210 ± .0949	-0.4539 ± .0673	-0.1320 ± .0835	-0.4624 ± .0668	-0.0798 ± .0844	-0.4094 ± .0707	-0.0774 ± .0845	-0.3294
	$r R SO_4$	-0.0867 ± .0642	-0.2894 ± .0778	-0.3197 ± .0763	-0.2358 ± .0802	-0.3226 ± .0761	-0.2928 ± .0815	-0.1296 ± .0836	-0.2346 ± .0774	-0.2547 ± .0795	-0.3027 ± .0772	-0.2569
Chloride and—	$r Cl \Delta$	+0.2512 ± .0844	+0.1602 ± .0878	+0.2644 ± .0817	+0.2468 ± .0832	+0.3588 ± .0740	+0.2536 ± .0797	+0.3414 ± .0776	+0.4344 ± .0718	+0.3040 ± .0675	+0.3008 ± .0779	+0.2828
	$r Cl \kappa$	+0.1684 ± .0892	+0.1047 ± .0900	+0.3303 ± .0796	+0.1023 ± .0876	+0.3120 ± .0763	+0.0833 ± .0849	+0.2438 ± .0640	+0.0836 ± .0859	+0.2714 ± .0675	+0.2714 ± .0875	+0.0970
	$r Cl Cl$	+0.0882 ± .0881	+0.1163 ± .0882	+0.1145 ± .0882	+0.0490 ± .0876	+0.2725 ± .0817	+0.0817 ± .0817	+0.3006 ± .0812	+0.2020 ± .0842	+0.2304 ± .0714	+0.2304 ± .0842	+0.0832
	$r Cl SO_4$	+0.2600 ± .0830	+0.0816 ± .0894	+0.2676 ± .0820	+0.2362 ± .0828	+0.2306 ± .0835	+0.0875 ± .0843	+0.186 ± .0865	+0.1240 ± .0865	+0.2190 ± .0865	+0.1354 ± .0865	+0.1354
Sulphate and—	$r SO_4 \Delta$	+0.2848 ± .0828	+0.2540 ± .0851	+0.5108 ± .0819	+0.0707 ± .0891	+0.4812 ± .0831	+0.0941 ± .0849	+0.4620 ± .0849	+0.1114 ± .0875	+0.4347 ± .0875	+0.4347 ± .0875	+0.1326
	$r SO_4 \kappa$	+0.1870 ± .0886	+0.0908 ± .0908	+0.5723 ± .0601	+0.0430 ± .0884	+0.3924 ± .0737	+0.0044 ± .0856	+0.2342 ± .0830	+0.1092 ± .0875	+0.3440 ± .0875	+0.3440 ± .0875	+0.0086
	$r SO_4 Cl$	+0.2453 ± .0847	+0.0069 ± .0901	+0.2131 ± .0838	-0.1354 ± .0862	+0.2078 ± .0813	-0.2053 ± .0814	+0.3315 ± .0782	-0.1707 ± .0852	+0.2495 ± .0852	+0.2495 ± .0852	+0.1295
	$r SO_4 SO_4$	+0.2160 ± .0859	+0.1100 ± .0880	+0.2632 ± .0803	+0.1704 ± .0853	+0.2283 ± .0805	+0.1291 ± .0836	+0.1635 ± .0855	+0.1482 ± .0859	+0.2253 ± .0859	+0.2253 ± .0859	+0.1394

* Depth of soil sample.

^b Calculated from the average values of the individual determinations of the first to fourth foot samples, inclusive.

TABLE 2.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity (electrical resistance, chloride content, and sulphate content) and various tissue-fluid properties in Pima Egyptian and Lone Star upland cotton grown at the United States Field Station, Sacoan, Ariz., in 1923

[Experiment 1/23; series 2-A, determinations based on plants grown on the entire plot]

Variables	Correlation	Coefficient for—										Average correlation coefficient	
		First foot ^a		Second foot ^a		Third foot ^a		Fourth foot ^a		All layers ^b			
		Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland		
Resistance and—	Δ	-0.4126± .0670	-0.4608± .0664	-0.5194± .0616	-0.4338± .0684	-0.4374± .0682	-0.4697± .0957	-0.4719± .0655	-0.4461± .0675	-0.5240± .0612	-0.5205± .0615	-0.4603 -0.4526	
	κ	-0.4743± .0653	-0.3478± .0741	-0.4500± .0672	-0.3228± .0755	-0.4823± .0647	-0.3544± .0737	-0.5062± .0627	-0.3046± .0765	-0.5397± .0579	-0.3912± .0714	-0.4782 -0.3324	
	Cl.....	-0.1483± .0824	-0.2706± .0781	-0.3063± .0764	-0.2247± .0800	-0.4849± .0645	-0.2638± .0784	-0.4996± .0633	-0.2132± .0805	-0.4314± .0686	-0.2773± .0778	-0.3596 -0.2431	
	SO ₄	-0.1935± .0812	-0.3445± .0743	-0.3140± .0760	-0.3611± .0733	-0.2024± .0808	-0.3292± .0752	-0.3449± .0743	-0.3862± .0717	-0.2986± .0768	-0.3949± .0712	-0.2637 -0.3552	
Chloride and—	Δ	+0.4491± .0733	+0.4323± .0746	+0.5745± .0583	+0.5283± .0628	+0.6579± .0482	+0.7354± .0390	+0.7313± .0408	+0.8134± .0297	+0.8134± .0297	+0.8134± .0297	+0.6032 +0.6274	
	κ	+0.3547± .0802	+0.2473± .0862	+0.4791± .0671	+0.4978± .0655	+0.4898± .0651	+0.5417± .0900	+0.6132± .0546	+0.6458± .0512	+0.6458± .0512	+0.6458± .0512	+0.4832 +0.4831	
	Cl.....	+0.2737± .0849	+0.3125± .0828	+0.2025± .0835	+0.0699± .0863	+0.2802± .0783	+0.4262± .0695	+0.3828± .0749	+0.4010± .0737	+0.3828± .0749	+0.4010± .0737	+0.2848 +0.3062	
	SO ₄	+0.1632± .0893	+0.2619± .0835	+0.4082± .0768	+0.3430± .0726	+0.3171± .0764	+0.3293± .0753	+0.4610± .0660	+0.4610± .0660	+0.4610± .0660	+0.4610± .0660	+0.3303 +0.3628	
Sulphate and—	Δ	+0.1076± .0808	+0.3946± .0715	+0.5138± .0641	+0.4353± .0706	+0.3793± .0728	+0.3263± .0736	+0.3950± .0741	+0.2898± .0904	+0.2898± .0904	+0.2898± .0904	+0.3489 +0.3620	
	κ	+0.2737± .0808	+0.3946± .0715	+0.5138± .0641	+0.4353± .0706	+0.3793± .0728	+0.3263± .0736	+0.3950± .0741	+0.2898± .0904	+0.2898± .0904	+0.2898± .0904	+0.3172 +0.1899	
	Cl.....	+0.1179± .0905	+0.3738± .0719	+0.2025± .0835	+0.2427± .0820	+0.2802± .0780	+0.4262± .0695	+0.3828± .0749	+0.4010± .0737	+0.3828± .0749	+0.4010± .0737	+0.2108 +0.2357	
	SO ₄	+0.2559± .0838	+0.2713± .0864	+0.3511± .0763	+0.4045± .0740	+0.1760± .0822	+0.1986± .0835	+0.2007± .0643	+0.2613± .0618	+0.2613± .0618	+0.2613± .0618	+0.2467 +0.2318	

^a Depth of soil sample.

^b Calculated from the average values of the individual determinations of the first to fourth foot samples, inclusive.

TABLE 3.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity (electrical resistance, chloride content, and sulphate content) and various tissue-fluid properties in Pima Egyptian and Love Star upland cotton grown at the United States Field Station, Sacaton, Ariz., in 1923

[Experiment 1/23, series 2-B, determinations based on plants grown on south half of plot]

Variables	Correlation	Coefficient for—										Average correlation coefficient	
		First foot ^a		Second foot ^a		Third foot ^a		Fourth foot ^a		All layers ^b			
		Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland
Resistance and—	<i>r</i> Δ	-0.1492± .0858	-0.2346± .0830	-0.3686± .0759	-0.1843± .0548	-0.1711± .0852	-0.2175± .0836	-0.2448± .0825	-0.1734± .0852	-0.2669± .0816	-0.2424± .0826	-0.2334	-0.2025
	<i>r</i> κ	-0.0034± .0878	-0.1038± .0869	-0.0031± .0878	-0.0211± .0878	-0.0032± .0878	-0.0422± .0876	-0.0036± .0878	-0.0567± .0875	-0.0041± .0878	-0.0443± .0876	-0.0034	-0.0560
	<i>r</i> Cl	-0.0539± .0876	-0.0482± .0876	-0.2523± .0822	-0.0099± .0878	-0.4367± .0865	-0.0504± .0875	-0.4083± .0860	-0.0091± .0878	-0.3877± .0746	-0.0312± .0877	-0.3078	-0.0241
	<i>r</i> SO ₄	+0.0607± .0875	-0.1988± .0843	-0.1312± .0863	-0.2574± .0829	+0.0423± .0876	-0.1723± .0852	-0.1634± .0854	-0.2471± .0824	-0.0498± .0876	-0.2321± .0831	-0.0484	-0.2139
Chloride and—	<i>r</i> Cl Δ	+0.2268± .0900	+0.1433± .0934	+0.3642± .0789	+0.2163± .0867	+0.2170± .0824	+0.2848± .0807	+0.2151± .0875	+0.3202± .0824	+0.2658	+0.2658	+0.2658	+0.2411
	<i>r</i> Cl κ	+0.2127± .0911	+0.0023± .0854	+0.1788± .0880	+0.0455± .0907	+0.1573± .0856	-0.0335± .0876	+0.1639± .0893	-0.0648± .0914	+0.1782	+0.1782	+0.1782	-0.0169
	<i>r</i> Cl Cl	+0.2337± .0902	+0.0003± .0854	+0.0912± .0902	-0.1341± .0883	+0.3278± .0781	+0.0139± .0878	+0.3720± .0791	-0.0025± .0918	+0.2562	+0.2562	+0.2562	-0.0305
	<i>r</i> Cl SO ₄	-0.1885± .0920	+0.0465± .0852	+0.1869± .0880	+0.2801± .0838	+0.0022± .0878	+0.1768± .0851	+0.1570± .0895	+0.1497± .0897	+0.0379	+0.0379	+0.0379	+0.1633
Sulphate and—	<i>r</i> SO ₄ Δ	+0.0774± .0848	+0.2660± .0886	+0.2790± .0839	+0.0710± .0905	+0.1646± .0854	+0.0055± .0878	+0.3532± .0803	+0.1312± .0902	+0.2185	+0.2185	+0.2185	+0.1184
	<i>r</i> SO ₄ κ	+0.3885± .0910	+0.0333± .0833	+0.1269± .0864	-0.1511± .0889	+0.2532± .0834	-0.2033± .0842	+0.1542± .0896	-0.1418± .0899	+0.2289	+0.2289	+0.2289	-0.1157
	<i>r</i> SO ₄ Cl	+0.1394± .0931	-0.0290± .0853	+0.1289± .0896	-0.0012± .0910	+0.2337± .0830	-0.1100± .0867	-0.2617± .0855	+0.0240± .0917	+0.1932	+0.1932	+0.1932	-0.0290
	<i>r</i> SO ₄ SO ₄	+0.0222± .0853	+0.1507± .0832	+0.1726± .0862	+0.1986± .0896	+0.0253± .0878	-0.0030± .0878	+0.0899± .0910	+0.1972± .0882	+0.0777	+0.0777	+0.0777	-0.1162

^a Depth of soil sample. Calculated from the average values of the individual determinations of the first to fourth foot samples, inclusive

The data may be most readily understood if presented graphically as in Figure 1 for the first series and in Figures 2 and 3 for the second series of determinations. Figure 3 contains determinations for only the south half of the plot and is therefore quite comparable with Figure 1. Figure 2 represents correlations based on the total material from the less saline south half of the experimental area and on the few

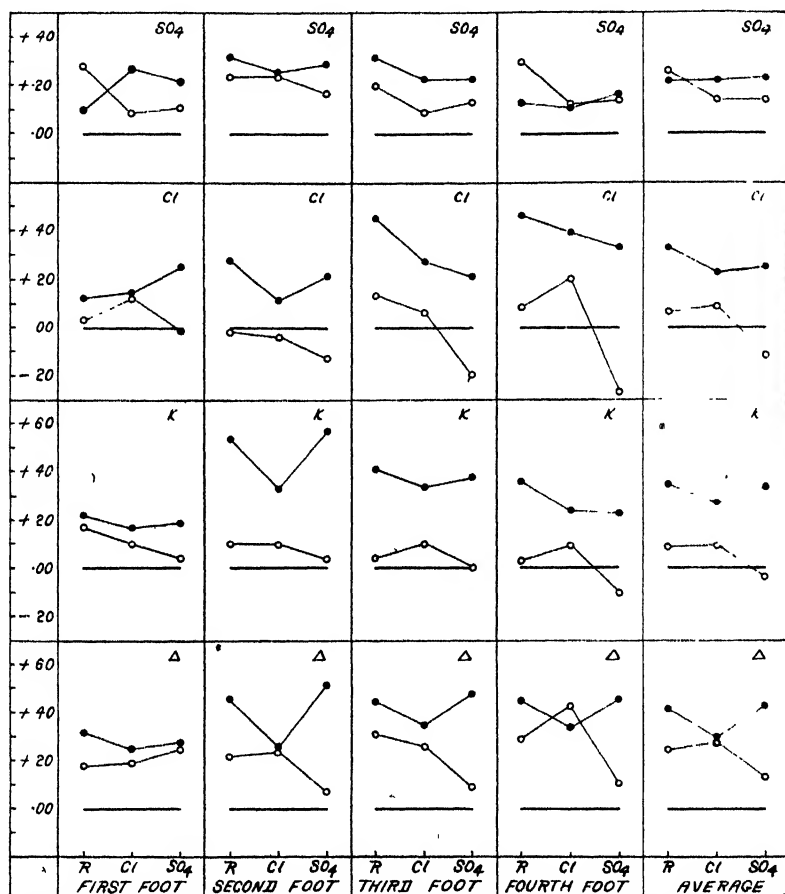


FIGURE 1.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity and various tissue-fluid properties in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23). First series of determinations, based on south half of plot

available determinations from the highly saline north half of the experimental tract.

In these figures the magnitudes of the correlation coefficients are represented on the scale of ordinates. With the exceptions the correlations between soil resistance and the tissue-fluid properties, as given in the table of constants, have negative signs. Since soil salinity is measured in terms of soil resistance and is therefore, within limits, inversely proportional to the resistance, a negative coefficient indicates that higher soil salinities are associated with higher values of osmotic

concentration and electrical conductivity and higher chloride and sulphate contents of the tissue fluids. In representing these correlations in connection with the others the sign has therefore been changed in order to facilitate comparisons between the several different relationships considered and to reduce the vertical dimensions of the diagrams to a size practicable for publication.

In Figures 1 to 3 the soil property involved is indicated on the axis of abscissas, at the bottom of the vertical sections of panels, as R ,

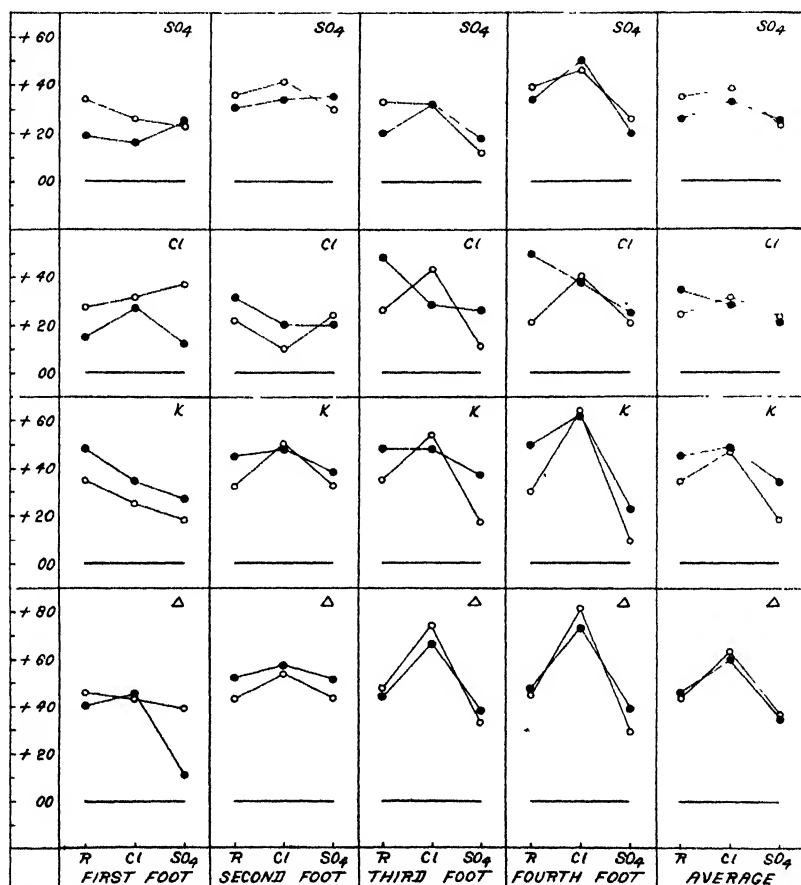


FIGURE 2—Correlation coefficients measuring the relationship between each of the three measures of soil salinity and various tissue-fluid properties in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23). Second series of determinations, based on entire plot

Cl , and SO_4 for the first, second, third, and fourth foot. The right-hand sections of the figures represent the average magnitudes of the correlation coefficients (not the correlation coefficients for the averages of the soil properties) for the four different soil layers and the plant characteristics.

Figures 1 to 3 are divided into four transverse sections, each showing the relationships between a given property of the soil (R , Cl , or SO_4) and the osmotic concentration as measured in terms of freezing-

point depression (Δ), specific electrical conductivity (κ), chloride content (Cl), and sulphate content (SO_4) of the tissue fluids. The correlation coefficients for Pima Egyptian cotton are represented by the position of solid dots, while those for Lone Star upland cotton are shown by the position of circles, on the scale of ordinates. The position of these dots and circles with reference to the transverse bars that

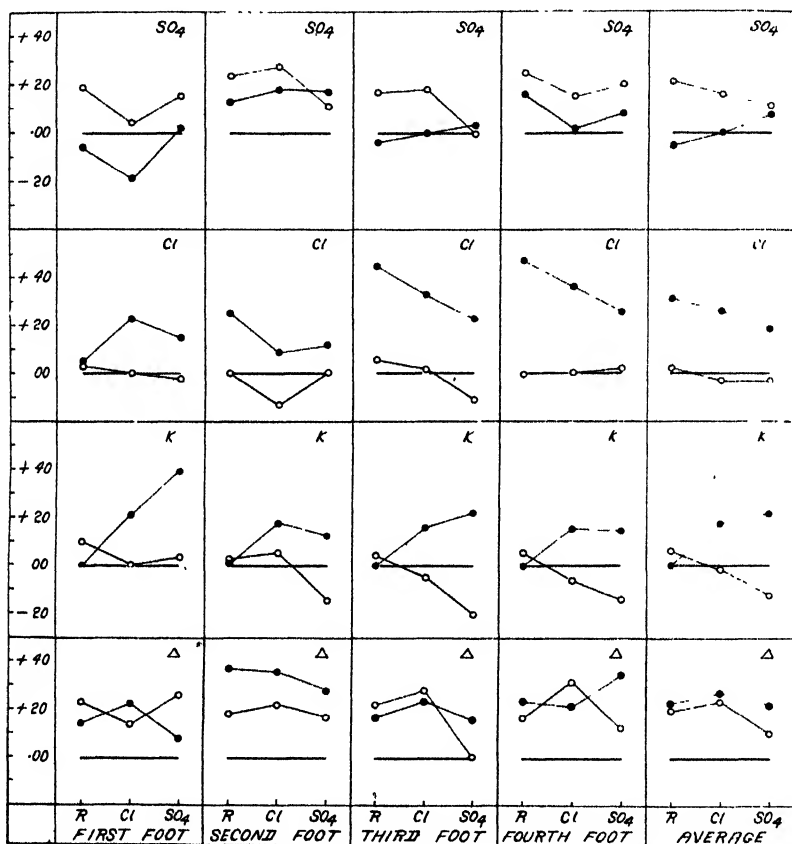


FIGURE 3.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity and various tissue-fluid properties in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23). Second series of determinations, based on south half of plot

represent zero correlation will make the signs and the magnitudes of the coefficients quite clear.

Considering first the relationship between each of the three measures of soil salinity and the freezing-point depression (Δ) of the leaf-tissue fluids, as shown in the lowest transverse section of Figures 1 to 3, it appears that while the coefficients are distributed with some irregularity, all are positive values. It is interesting to note that in 25 of the 36 constants, exclusive of averages, the coefficient for Pima Egyptian cotton is higher than that for Lone Star upland cotton, whereas in 11 of the cases the reverse is true. This point will be considered later.

Inspection of the transverse section, second from the bottom (figs. 1 to 3), representing the relationship between the properties of the soil and the specific electrical conductivity (κ) of the tissue fluids, shows that, in general, there is a positive correlation between the measures of the electrolyte content of the soil and the associated electrolyte content of the plant-tissue fluids.

In the first series of determinations (fig. 1) the coefficients for Pima Egyptian cotton are conspicuously higher than those for Lone Star upland cotton. As a matter of fact, the latter deviate only slightly from zero correlations. Their general average is actually negative in sign in the case of the relationship between the sulphate content of the soil and the electrical conductivity of the tissue fluids. If the second series of determinations is limited to the same area as the first (fig. 3), essentially the same results will be found, although it is to be noted that the correlations for soil resistance and the specific electrical conductivity of the tissue fluids of Pima Egyptian cotton are sensibly 0 for all levels, while the correlations for Lone Star upland cotton are only slightly positive. If the entire area of the experimental plot is included (fig. 2) it appears that all the coefficients are conspicuously positive and of the general order of magnitude $r=0.40$. These relationships will be considered in greater detail after the correlations for the two individual electrolytes of the tissue fluids are discussed.

The relationships between soil salinity and the chloride content (\mathcal{C}) of the leaf-tissue fluids are of especial interest since the Egyptian and the upland cottons have been shown to be differentiated with respect to chloride content (7, 11, 12, 13) and sulphate content (7, 10, 13). In the 36 determinations of both the first and second series, the correlations between the three measures of the concentration of the soil solution and the chloride content of the leaf-tissue fluids based on the Egyptian plants are positive. In the first series of determinations (fig. 1) and in those determinations of the second series that are based on the less saline area of the field (fig. 3), the correlations for the Egyptian cotton are higher in all cases than those for the upland variety. This is not uniformly the case if the correlations include those determinations of the second series that are based on the more saline area of the field (fig. 2).

While the correlations between the three measures of soil salinity and the chloride content of the Egyptian variety are positive in all cases, this condition is not found to prevail for the upland variety, for which a number of the coefficients are essentially 0 or even negative in sign. While great weight should not be put on these results, they nevertheless furnish a strong suggestion that the Egyptian type not only absorbs larger quantities of chlorides (as shown in previous investigations) than does the upland type, but that it is more exactly adjusted to the chloride content of the substratum than is the upland type of cotton. It may be urged in criticism of this view that only one-third of these coefficients measure the relationship between the chloride content of the soil and the chloride content of the leaf-tissue fluids, while the others measure the relationship between the electrical resistance and sulphate content of the soil on the one hand and the chloride content of the leaf-tissue fluids on the other. In reply to this suggestion, it must be pointed

out that the electrical resistance of the soil is in part inversely proportional to its chloride content and that there is in all probability a correlation between the chloride content and the sulphate content of the individual areas of the field. Thus a full solution of the problem under consideration involves a study of the correlations between each soil property and all the other soil properties as well as of those between the soil properties on the one hand and tissue-fluid properties on the other. It has not yet been possible to undertake this special investigation.

Finally, the relationship between the three measures of soil salinity and the sulphate content (SO_4) of the leaf-tissue fluids must be considered.

Figure 1 shows that for the first series of determinations the coefficients are positive in all cases. For the second series of determinations based on the same area (fig. 3) this is also generally true. In the latter case, however, it is to be noted that a number of the determinations for Egyptian cotton fall near or even below the line denoting zero correlation. When the correlations include determinations based on plants from the more saline portion of the field (fig. 2) all the coefficients are positive and of the order of magnitude $r=0.10$ to 0.50 .

In comparing the magnitudes of the correlations for sulphate content in the Egyptian and upland types, it is to be noted that in only 16 out of the 36 cases is the correlation for the Egyptian variety higher than that for the upland variety. In 20 of the cases the reverse is true.⁴

While the differences between the correlations for the sulphate content of the Egyptian and upland type are not large, it is to be noted that they are the reverse of those found for the correlations for the chloride content of the tissue fluids. The results suggest that the correlation between the soil salinity and sulphate content may be higher in the upland than in the Egyptian type. This must, however, remain merely a suggestion until further data are available.

Taking all the foregoing results as a whole, they show clearly that it is quite possible to deal in a quantitative way with the relationships between the analytically determined values of the ions of the soil and the characteristics of the plants when the latter are grown under ordinary field conditions. While the correlations are low in magnitude and somewhat irregular in distribution, this is unquestionably in part due to the relatively rough methods employed in all stages of this preliminary investigation. When methods of sampling and of the determination of the soil properties are improved, data of a much higher degree of precision may be secured.

The preceding discussion has been limited to a consideration of the general order of magnitude of the coefficients measuring the relationships between the properties of the soil and the physicochemical properties of the leaf-tissue fluids in the two varieties. It is now necessary to consider the relative magnitudes of the several classes of correlations. Two groups of comparisons are necessary for a full understanding of the results.

⁴ The differences in the coefficients are so slight in some cases that they can not be read from the diagrams. They have, therefore, been summarized from Tables 1-3. In the correlations for soil resistance the negative signs have been made positive for purposes of comparison.

(1) The differences

$'R_{\Delta} - 'R_{\kappa}$	$'Cl_{\Delta} - 'Cl_{\kappa}$	$'SO_{4\Delta} - 'SO_{4\kappa}$
$'R_{\Delta} - 'R_{Cl}$	$'Cl_{\Delta} - 'Cl_{Cl}$	$'SO_{4\Delta} - 'SO_{4Cl}$
$'R_{\Delta} - 'R_{SO_4}$	$'Cl_{\Delta} - 'Cl_{SO_4}$	$'SO_{4\Delta} - 'SO_{4SO_4}$
$'R_{\kappa} - 'R_{Cl}$	$'Cl_{\kappa} - 'Cl_{Cl}$	$'SO_{4\kappa} - 'SO_{4Cl}$
$'R_{\kappa} - 'R_{SO_4}$	$'Cl_{\kappa} - 'Cl_{SO_4}$	$'SO_{4\kappa} - 'SO_{4SO_4}$
$'R_{Cl} - 'R_{SO_4}$	$'Cl_{Cl} - 'Cl_{SO_4}$	$'SO_{4Cl} - 'SO_{4SO_4}$

show the relative magnitudes of the correlations between each of the tissue-fluid properties and each of the three measures of the concentration of the soil solution.

(2) The differences

$'R_{\Delta} - 'Cl_{\Delta}$	$'R_{\kappa} - 'Cl_{\kappa}$	$'R_{Cl} - 'Cl_{Cl}$	$'R_{SO_4} - 'Cl_{SO_4}$
$'R_{\Delta} - 'SO_{4\Delta}$	$'R_{\kappa} - 'SO_{4\kappa}$	$'R_{Cl} - 'SO_{4Cl}$	$'R_{SO_4} - 'SO_{4SO_4}$
$'Cl_{\Delta} - 'SO_{4\Delta}$	$'Cl_{\kappa} - 'SO_{4\kappa}$	$'Cl_{Cl} - 'SO_{4Cl}$	$'Cl_{SO_4} - 'SO_{4SO_4}$

show the relative magnitudes of the correlations between each of the three measures of the concentration of the soil solution and each of the tissue-fluid properties.

The numerical values of all these differences and the probable errors which are necessary for determining their significance may be calculated from the constants in Tables 1 to 3. Since the probable errors of the correlation coefficients are for the most part relatively large because of the generally low value of the coefficients and the relatively small number of determinations upon which they are based, it does not seem desirable to present and discuss in detail the numerical values. The comparisons may be made at a saving of space by a graphic presentation of the differences. (Fig. 4.)

COMPARISON OF CORRELATIONS BETWEEN DIFFERENT TISSUE-FLUID PROPERTIES AND A GIVEN MEASURE OF SOIL SALINITY

In Figure 4 the two coefficients involved are represented on the 18 panels, each of which presents one set of differences. The comparison is between the values of the coefficients given on the lower margin of the panel and that given above the scale representing the depth of the sample. The differences are taken as shown in the first of the two schemes of differences above. The reader will find it convenient to note that the value of the coefficient indicated above the scale of depth is always subtracted from that of the coefficient given on the basal margin of the panel. Thus when the correlation indicated by the marginal symbols has a larger value (algebraically considered, and regarding the reversal of the signs which has been made in the correlations involving soil resistance), the differences (scale of ordinates) will lie above the bar denoting a difference of zero (or, for brevity, the zero bar).

In each of the panels, three scales represent the correlations for the soil samples taken at the first to the fourth foot (1 to 4) and the average correlations (A). The three series of correlations determined are represented by 1, 2-A, and 2-B, as defined on p. 770.

A comparison of the correlations between the electrical resistance of the soil solution and the osmotic concentration (as measured in terms of the freezing-point depression) of the leaf-tissue fluids ($'R_{\Delta}$) with the correlations between soil resistance and the specific electrical conductivity of the leaf-tissue fluids ($'R_{\kappa}$) shows that, for the Egyptian cotton, the differences are practically 0 in series 1 and 2-A. In

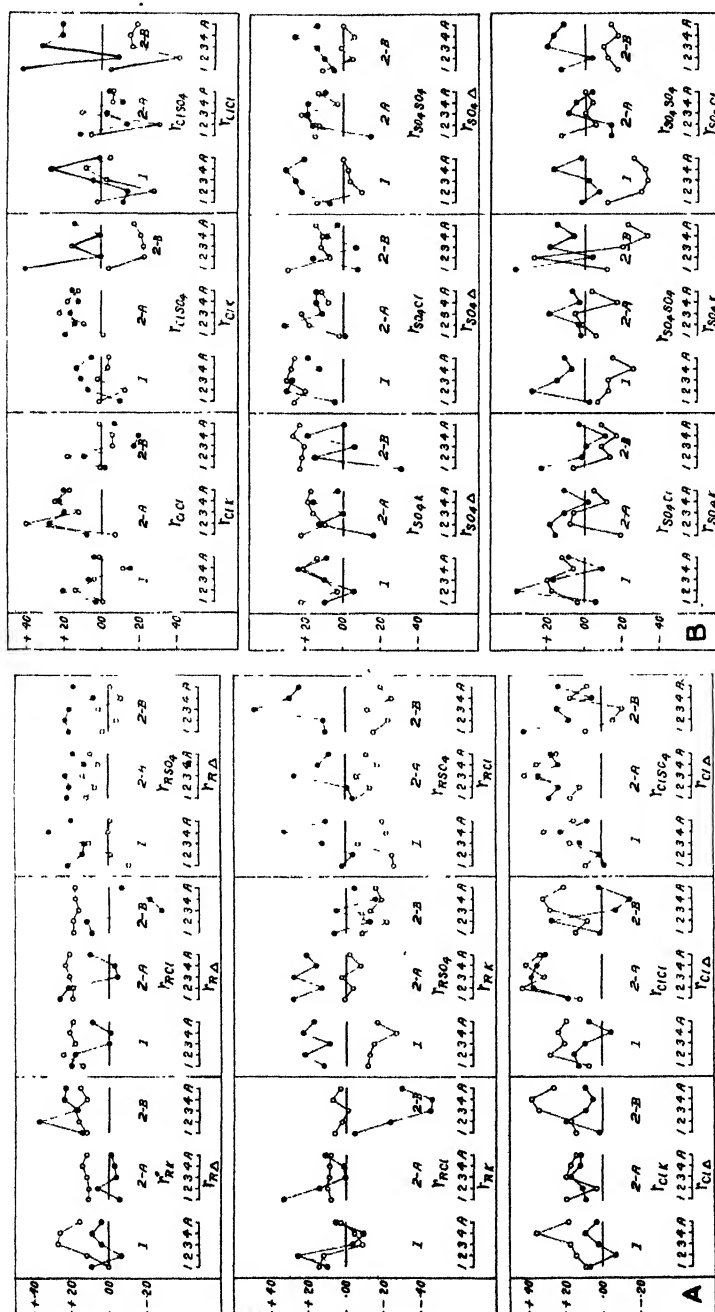


FIGURE 4.—Differences between correlations of various tissue-fluid properties (Series 1, 2-A, and 2-B) with a given measure of soil salinity in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23)

series 2-B the Egyptian plants show higher correlations between soil resistance and osmotic concentration than between soil resistance and electrical conductivity ($'R\Delta > 'R\kappa$). For the upland cotton, the correlation between soil resistance and osmotic concentration is higher than that between soil resistance and electrical conductivity in all cases ($'R\Delta > 'R\kappa$).

The correlations between soil resistance and the concentration of all solutes (as measured by the freezing-point depression) in the leaf-tissue fluids are, for upland cotton, higher in all cases than the correlations between soil resistance and the concentration of chlorides in the leaf-tissue fluids ($'R\Delta > 'RCl$). For the Egyptian cotton the differences are in general much lower, and certain of them are well on the negative side ($'RSO_4 > 'R\Delta$).

The correlations between soil resistance and total solutes are higher in the Egyptian cotton than those between soil resistance and tissue-fluid sulphates ($'R\Delta > 'RSO_4$). The differences between these two correlations are smaller in the upland variety and sometimes on the negative side ($'R\Delta_4 > 'RSO_4$).

In the upland variety $'R\kappa > 'RCl$ in the majority of cases. The differences are, however, so small that little stress is to be laid on them. In series 2-B the values of $'RCl$ for Egyptian cotton seem to be distinctly larger than those of $'R\kappa$.

It appears that in some cases $'R\kappa > 'RSO_4$, whereas in other cases $'R\kappa < 'RSO_4$ in both Egyptian and upland cotton.

The third panel of the central row of Figure 4, A, shows that in the Egyptian variety the correlation between soil resistance and tissue-fluid chlorides is generally greater than that between soil resistance and tissue-fluid sulphates ($'RCl > 'RSO_4$), whereas in the upland variety the reverse ($'RSO_4 > 'RCl$) is invariably true.

The three lower panels of Figure 4, A, and the three upper panels of Figure 4, B, give the results of comparisons of the correlations between the chloride content of the soil and each of the properties of the leaf-tissue fluids.

A comparison of the correlation of the chloride content of the soil and the total solutes of the leaf-tissue fluids as measured in terms of freezing-point depression, $'Cl\Delta$, and the chloride content of the soil and the specific electrical conductivity of the leaf-tissue fluids, $'Cl\kappa$, shows that, in general, in both Egyptian and upland cotton $'Cl\Delta > 'Cl\kappa$.

These results are substantiated in a general way by the comparisons of the correlations between the chloride content of the soil and the total solutes of the leaf-tissue fluids ($'Cl\Delta$) with those between the chloride content of the soil and the concentration of chlorides ($'ClCl$) and sulphates ($'ClSO_4$) in the leaf-tissue fluids, as shown by the lower central and right-hand panels of Figure 4, A. Generally, although not invariably, $'Cl\Delta > 'ClCl$ and $'Cl\Delta > 'ClSO_4$ in both Egyptian and upland cotton.

A comparison of the correlations between the chloride content of the soil and the electrical resistance of the leaf-tissue fluids, $'Cl\kappa$, and the correlations between the chloride content of the soil and the chloride content of the leaf-tissue fluids, $'ClCl$ (fig. 4, B), shows such irregularities that any final conclusion is impossible. There is, however, some evidence that in both the Egyptian and upland varieties

${}^{\circ}Cl_k > {}^{\circ}ClCl$. In the comparison between ${}^{\circ}Cl_k$ and ${}^{\circ}ClSO_4$ it appears that in general ${}^{\circ}Cl_k > {}^{\circ}ClSO_4$ in the Egyptian variety, whereas the differences are insignificant in the upland variety. In the comparison of ${}^{\circ}ClSO_4$ with ${}^{\circ}ClCl$, the differences are so evenly distributed about 0 that no final conclusion is to be drawn concerning any differences in these coefficients.

Comparisons of the correlations between the sulphate content of the soil and each of the various tissue-fluid properties of the plant are made in the six lower panels of Figure 4, B. The results from a comparison of ${}^{\circ}SO_4\Delta$ with the other tissue-fluid properties show that in all cases ${}^{\circ}SO_4\Delta > {}^{\circ}SO_4k$ for the upland variety. This condition is not realized in the case of the Egyptian type. In the upland varieties the relation ${}^{\circ}SO_4\Delta > {}^{\circ}SO_4Cl$ is found in all cases. In the Egyptian type this condition is generally but not invariably realized. Finally the relation ${}^{\circ}SO_4\Delta > {}^{\circ}SO_4SO_4$ is found in all but one case in the Egyptian type. This condition is not realized in the upland type.

The comparison between ${}^{\circ}SO_4k$, on the one hand, and ${}^{\circ}SO_4Cl$ and ${}^{\circ}SO_4SO_4$ on the other, and between ${}^{\circ}SO_4Cl$ and ${}^{\circ}SO_4SO_4$ shows results too irregular to merit further discussion until additional data are available.

Notwithstanding various irregularities (believed to be due primarily to limitations in the experiment, which will be discussed later), there seem to be certain definite trends in the results which are important in confirming earlier results and in suggesting further lines of work.

(1) In the upland variety the correlation between the electrical resistance of the soil and the concentration of all solutes of the leaf-tissue fluids (as measured by freezing-point depression) is higher than the correlation between the salinity of the soil and the conductivity of the tissue fluids. The relationship is not so marked in the Egyptian type.

(2) In general the correlation between the analytically determined concentration of the two ions studied in the soils and the total solutes of the tissue fluids as measured in terms of osmotic concentration is higher than that between these ions and the various measures of electrolyte content of the tissue fluids.

(3) In the upland variety the correlations between the soil resistance and total solutes of the tissue fluids are higher than those between soil resistance and the chlorides of the tissue fluids (${}^{\circ}R\Delta > {}^{\circ}RCl$), whereas in the Egyptian variety the reverse (${}^{\circ}RCl > {}^{\circ}R\Delta$) may be true.

(4) In the Egyptian variety the correlations between soil resistance and total solutes are higher than those between soil resistance and tissue-fluid sulphate content (${}^{\circ}R\Delta > {}^{\circ}RSO_4$). The differences between these two correlations are smaller in the upland type, and their sign may in some cases be reversed (${}^{\circ}RSO_4 > {}^{\circ}R\Delta$).

(5) Paragraphs 3 and 4 suggest that the correlations between soil salinity and chloride content are higher in the Egyptian variety, whereas the correlations between soil resistance and sulphate content are higher in the upland type. Thus, there seem to be differences between the relationships of the specific ions of the leaf-tissue fluids of Egyptian and upland cotton to the concentration of the electrolytes in the soil as measured in terms of the electrical resistance.

COMPARISON OF CORRELATIONS BETWEEN DIFFERENT MEASURES OF SOIL SALINITY AND A GIVEN TISSUE-FLUID PROPERTY

The second group of comparisons—those showing the differences in the correlations between a given constituent of the leaf-tissue fluids and different measures of soil salinity—are represented graphically in Figure 5, which is constructed on the same principle as Figure 4.

The differences are so taken that when the correlation between the constants given at the lower margin of the panel is higher than that between the constants given above the scale for soil depth, the difference will lie above the bars denoting zero.⁵

Considering all the differences shown in the 12 panels of Figure 5, it appears that they are about equally distributed above and below the zero bars. Thus it must be recognized at the outset that there are no great differences between these pairs of correlations.

An examination of the correlations between soil resistance and tissue-fluid properties in comparison with those between soil chloride content and tissue-fluid properties shows that for freezing-point depression (Δ), specific electrical conductivity (κ), chloride content (Cl), and sulphate content (SO_4), the correlation between soil resistance and the given tissue-fluid property is sometimes higher and sometimes lower than that between soil chloride content and the same tissue-fluid property. A comparison of the correlations between the resistance of the soil and the tissue-fluid properties with the correlations between the sulphate content of the soil and the tissue-fluid properties shows similar results.

Thus it is quite clear that so far as the data of this investigation may serve as a basis for generalization, the electrical resistance of the soil is about as satisfactory a measure of salinity as analytically determined values of either sulphate or chloride content.

Comparison of the correlations between the tissue-fluid properties and the chloride and sulphate contents of the soil shows that for the relationships as a whole the differences are sometimes on the positive and sometimes on the negative side of 0. In short, the tissue-fluid properties are in some cases more closely correlated with the chloride content and in other cases more closely correlated with the sulphate content of the soil.

Since these latter differences are of particular interest because of the fact that the absorption of chlorides and sulphates has been shown to be differential for the two varieties, they may be examined in somewhat greater detail. The upper right-hand panel of Figure 5, A, shows that in upland cotton the correlation between the chlorides of the soil and the freezing-point depression of the tissue fluids is generally higher than that between the sulphate content of the soil and the freezing-point depression of the tissue fluids. The reverse relationship is, however, found in the first series of determination for Egyptian cotton. For the relationship between the chloride and sulphate content of the soil and the electrical conductivity of the tissue fluids, $'Cl\kappa > 'SO_4\kappa$ in all but one case in the upland variety. There are, however, many exceptions to this rule in the case of the Egyptian variety.

The comparisons between $'ClCl$ and $'SO_4Cl$ and between $'ClSO_4$ and $'SO_4SO_4$, as shown in the two right-hand panels of the lower

⁵ In all cases in which electrical resistance serves as a measure of the concentration of the soil solution the signs of these coefficients have been changed before determining differences.

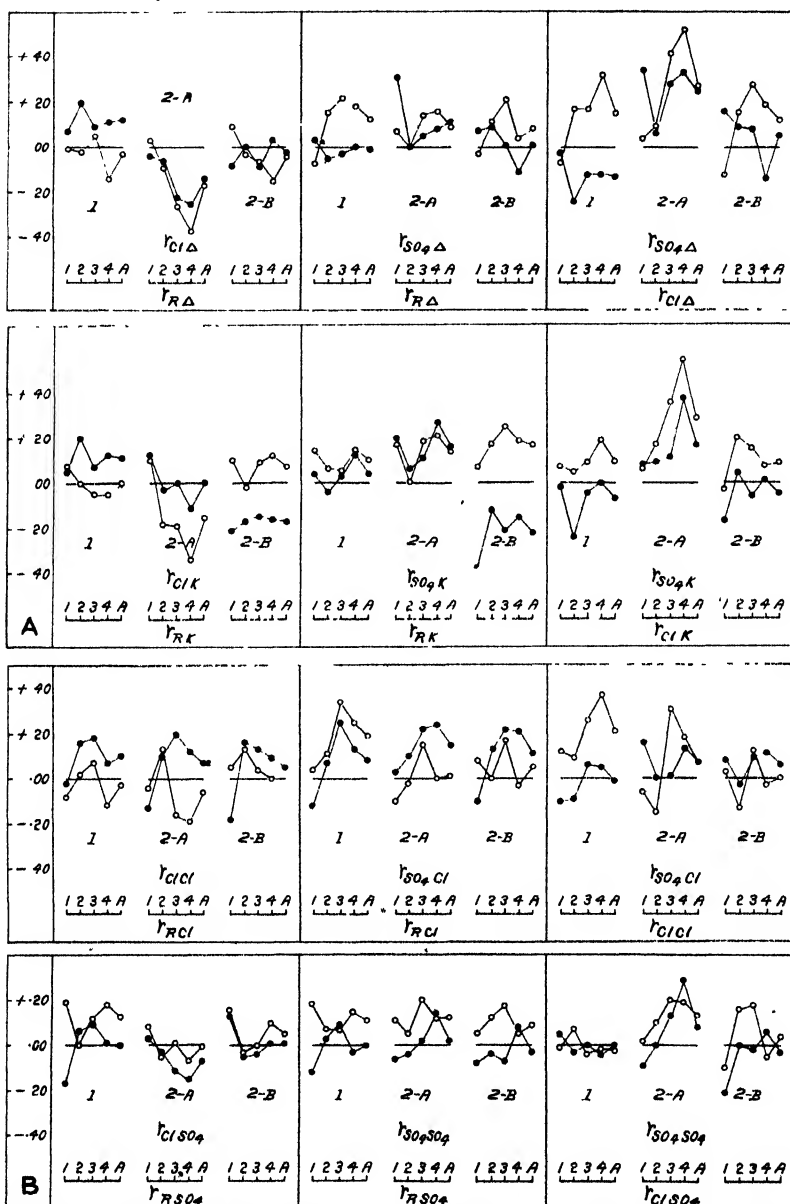


FIGURE 5.—Differences between correlations of a given tissue-fluid property (series 1, 2-A, and 2-B) with various measures of soil salinity in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23)

half of Figure 5, B, are of particular interest because they show that there are no conspicuous and universally constant differences between these pairs of correlations.

DISCUSSION

The results of the present investigation show that there is in general a positive correlation between the salinity of the soil and the osmotic concentration as measured in terms of freezing-point depression (Δ), specific electrical conductivity (κ), chloride content (Cl), and sulphate content (SO_4), of the leaf-tissue fluids of both Pima Egyptian and Lone Star upland cotton.

These relationships prevail whether soil salinity is measured in terms of electrical resistance (in which case the signs of the coefficients have been reversed, since salinity is, within limits, inversely proportional to electrical resistance) or as chloride content or sulphate content expressed as percentages of the air-dry weight of the soil.

Except for the lower numerical values of the coefficients, the results obtained in the present study fully confirm those of the earlier investigation on the tissue fluids of Pima Egyptian and Acala and Meade upland cotton (6). The writers are inclined to attribute the lower values of the coefficients obtained in the present investigation (based on experiment 1/23) as compared with the first study (based on experiment 3/22) to the lower technical quality of the data of the present series. This pertains especially to the manner in which the soil samples were taken. The limitations of the methods of sampling employed in experiment 1/23 have been adequately discussed in a study of the relationship between soil salinity and seedling stand (8), in which it was found that lower correlations prevailed in the experiment of 1923 than in that of 1922. The explanation of the lower numerical values of the coefficients is probably the same for seedling stand and for tissue-fluid properties.

The present results extend those already available by showing that the relationships between the properties of the soil and the characteristics of the plant hold for the analytically determined solutes (chlorides and sulphates) of the soil as well as for the more general measure of soil salinity provided by the electrical resistance of the soil mass.

Taking these results as a whole, it appears that the present data show no regular and universally valid difference between the correlations of any of the three measures of soil salinity and the tissue-fluid properties. There is unquestionably some correlation between each of these three measures of soil salinity and the various measures of concentration of solutes in the leaf-tissue fluids. It is not possible to assert, on the basis of the present results, that the correlations are universally higher for one measure of soil salinity than for another.

In any consideration of these results, the probability that the concentrations of the different salts in the soil are not independent but correlated must be borne in mind. This fact must have its bearing on the relationship between the electrical resistance of the soil and the tissue-fluid properties of the plant. Since electrical resistance depends upon all ions, it is not unreasonable to assume that it might give a higher correlation with tissue-fluid properties depending upon a number of solutes than would any one ion of the soil solution. This problem requires further investigation.

The foregoing explanation of the fact that the correlation between the electrical resistance of the soil and the tissue-fluid properties is equal to or greater than that between the specific ions of the soil and the tissue-fluid properties applies only to such general measures of tissue-fluid concentration as freezing-point depression and specific electrical conductivity. It can not be urged for such relations as those between the chloride content of the soil and the chloride content of the tissue fluids and between the sulphate content of the soil and the sulphate content of the tissue fluids in which other factors must be taken into consideration.

It is quite possible that the method of expressing the concentration of chloride and sulphate as a percentage of the dry weight of the soil, while conventional, is not the most advantageous for purposes of this kind. It would unquestionably be better if some measure of the concentration of these ions in the soil solution were made the basis of the correlations. Furthermore, it must be borne in mind that there may be large reserves of sulphate in the soil in the form of the relatively insoluble calcium sulphate. Thus the concentration of sulphate at any one moment in a water extract, expressed as a percentage of the weight of the soil, would not necessarily represent the conditions to which the plant itself is subjected.

It will be clear from the foregoing considerations that the problem of the relationship between the soil and the plant as it grows on the ordinary agricultural field is one of very great complexity. In the investigation of these interrelationships the correlations between the concentrations of the various constituents of the soils complicates the problem. More refined methods (both experimental and statistical) and larger series of data for both the soil and the plant will be necessary to unravel fully the tangle of interrelationships. Because of these obvious difficulties it seems best to defer a further statistical study of the problem until better data than those now in hand are available.

While the results secured up to the present time have an important bearing on the physiology of the cotton plant, they have a greater importance in relation to the methodology of agricultural investigation, in that they show that by a proper combination of agronomic, chemical, and biometric methods it will be possible to investigate in the field many problems which have been assumed to be open to investigation only under the controlled conditions of the laboratory.

The criticism may be made that the correlations in the present paper are of too low an order of magnitude to be taken as a trustworthy measure of the relationship between the variables of the soil and the plant. To this objection the reply is threefold.

(1) The assumption is tacitly made that in laboratory experimentation the results are of a high degree of trustworthiness. In the investigation of the relationship of the characters of the plant to the properties of the soil or culture medium this is by no means invariably the case. As far as the writers are aware it is still to be demonstrated that in the investigation of the relationship between the concentration of the solution and the characteristics of the plant results of a higher order of precision have been obtained in the laboratory than those which have been secured in these studies.

(2) There is no reason to assume that any individual interrelationship will be of a high order of magnitude. The determination of

the order of magnitude of relationships between soil properties and plant characters is, indeed, one of the objects of any investigation of this kind. It is the trustworthiness rather than the magnitude of the constants which is of importance in determining the value of the results.

(3) Various reasons for believing that the correlations for the present experiment were far from ideal have been indicated in the discussion. Much higher coefficients have been obtained for certain of the variables in an earlier investigation (6), which is believed to be technically better. Both this and the present study have been reconnaissance experiments. There is every reason to believe that the organization and technical details of such experiments can be materially improved, with the result that higher correlations and better fits of the regression lines or curves will be obtained.

SUMMARY

The present paper is one of a series dealing with the measurement of the relationships between the properties of the soil and the characteristics of the crop plant produced (6, 8, 9).

When suitable data are available the relationships between the characteristics of the plant and the salinity of the soil may be satisfactorily expressed in terms of the correlation coefficients. While salinity is an advantageous soil property for a first investigation, there is every reason to believe that the method may be extended to other soil properties.

The present paper confirms and extends the findings of the first study (6) of the relationship between the salinity of the soil and the tissue-fluid properties of Pima Egyptian, Meade upland, and Acala upland cotton in that positive correlations are demonstrated between the electrical conductivity, the chloride content, and the sulphate content of the soil, on the one hand, and the freezing-point depression, specific electrical conductivity, and chloride and sulphate content of the leaf-tissue fluids of the plant on the other. The coefficients obtained in the present study are lower than those found in the first investigation. The reasons for the differences are discussed.

The present findings extend those of the earlier investigation in that they are based on two additional variables for the soil (chloride content and sulphate content) and on an additional variable for the tissue fluids (sulphate content). The fact that statistically significant correlations have been obtained for all the combinations of the variables of the soil and the plant demonstrates the wide usefulness of the method of research employed.

The present study is admittedly preliminary in nature. It has fulfilled its purpose by confirming and extending previous results and by showing that physiological investigations of this kind may be carried out in conjunction with ordinary field-plot studies. When the refinements of technic that may be made at many points are available, results of far greater exactness and wider significance may be expected.

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NOTES ON THE HISTOLOGY OF THE ALMOND¹

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INTRODUCTION

In connection with a study of one of the technological problems involved in the salting of domestic almonds, some rather interesting data on the histology of the almond kernel or seed have been assembled. This material is presented here not only on account of its general interest to histologists, but because it may afford some assistance to the trade in developing a method for the determination of types if not of varieties of shelled almonds as they appear in the open market.

MATERIAL

The material studied² consists of four varieties of domestic almonds and four varieties of imported almonds. The domestic almonds, Nonpareil, Ne Plus Ultra, I. X. L., and Drake, are among the leading varieties grown in California, and the imported nuts, Etna, Marcona, Valencia, and Alicante, originally brought from Italy and Spain, are largely used by bakers and confectioners. The domestic nuts are paper and soft shelled, while the imported nuts are standard and hard shelled. On the basis of the hardness of the shell, these samples would be classed as follows:³

Paper.....	{	I. X. L.
		Ne Plus Ultra.
		Nonpareil.
Soft.....		Drake.
Standard.....		Marcona.
		Valencia.
Hard ---	{	Etna.
		Alicante (?).

The almond fruit is a typical drupe; that is, it consists of a single seed surrounded by a stony endocarp which forms the shell, a fleshy mesocarp, and an outer skin or epicarp. The mesocarp is tough and leathery and, with its protecting epicarp, splits away from the endocarp at maturity. The difference between hard and soft shelled almonds lies in the difference in texture of the endocarp. The shelled almond, or kernel, consists of the embryo, surrounded by the modified tissues of the ovule. The embryo itself is made up of a small, pointed radicle, a delicate plumule, and two straight massive cotyledons whose cells are closely packed with reserve food material in the form of oil droplets and protein granules. The surrounding tissues consist of the remnants of the endosperm and nucellus, and the integuments.

¹ Received for publication July 29, 1930, issued December, 1930.

² This material was furnished by the California Almond Growers' Exchange to whom the writer is greatly indebted.

³ Based on General Information on Almonds, Foodstuffs Division, Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce, 1923. [Mimeographed.]

REVIEW OF LITERATURE

Early work on the structure of the almond was done with a view to distinguishing microscopically between the kernels of almonds and such related seeds as peaches, apricots, and plums, which were being substituted for almonds, both in almond paste and in the expressing of the oil. Early workers on the microscopy of the almond seed agreed that there were no differences in structure between bitter and sweet almonds (1).⁴ The question of a difference between hard and soft shelled almonds seems not to have been raised until Young (13) contrasted them. His opening statement, however, is, "The bitter almond and the hard and soft or paper shelled varieties of the sweet almond all belong to one species and are hence very similar in structure." Young's work is the most detailed histological study of the almond published in English. Meyer (6) gives a careful description of the development of the seed from fertilization to maturity, with a discussion of the various tissues in the ripened seed and seed coat. Tschirch (10) bases his discussion on the best of the earlier workers and adds several new points, especially along the line of microchemical tests for cell-wall substances. Kracmer (5) offers probably the best summary in English of later foreign work, with well-executed line drawings, which are for the most part adaptations from other writers. Winton (11) seems to be the only other American writer who has published work on this subject.

METHOD

Preliminary examination having shown that in all probability the greatest anatomical differences between varieties or types of nuts were to be found in the "skin" or tissues surrounding the embryo, the structure of these tissues was worked out in some detail. Skins removed by blanching, or soaking for two or three minutes in boiling water, were boiled in 2 per cent potassium hydroxide and allowed to stand overnight, or until shrunk tissues and "obliterated cells" were sufficiently swelled so that their shape, size, arrangement, and relationships could be determined. Following this treatment, the aleurone layer could be stripped from the inner surface of the testa, the epidermal stone cells or hairs could be scraped from the outer surface with a dull scalpel, and the remaining tissues could be teased apart with needles or fine-pointed tweezers. Material was also embedded in paraffin, sectioned 10 microns thick, and stained with safranin and light green, using Land's schedule as given by Chamberlain (2). In addition to differentiating cellulose and lignin in the testa, this stain proved particularly satisfactory for the storage tissue of the cotyledons, the cell walls staining green and the protein granules bright red. Microchemical tests used were: For lignin, phloroglucinol-hydrochloric acid; for cellulose, chlorzinciodide; for cutin and oil, Sudan III; for tannin, ferric chloride; for protein, iodine in potassium iodide and the xanthoproteic reaction.

THE SEED COAT

The inner white layer stripped away from the brown or yellowish-brown testa after maceration in 2 per cent potassium hydroxide con-

⁴ References made by number (italic) to Literature Cited, p. 800.

sists almost entirely of aleurone and obliterated cells; that is, endosperm and nucellus or perisperm. The aleurone cells of the endosperm (fig. 1, A, 2, *al*) are thick walled, quadrilateral to hexagonal

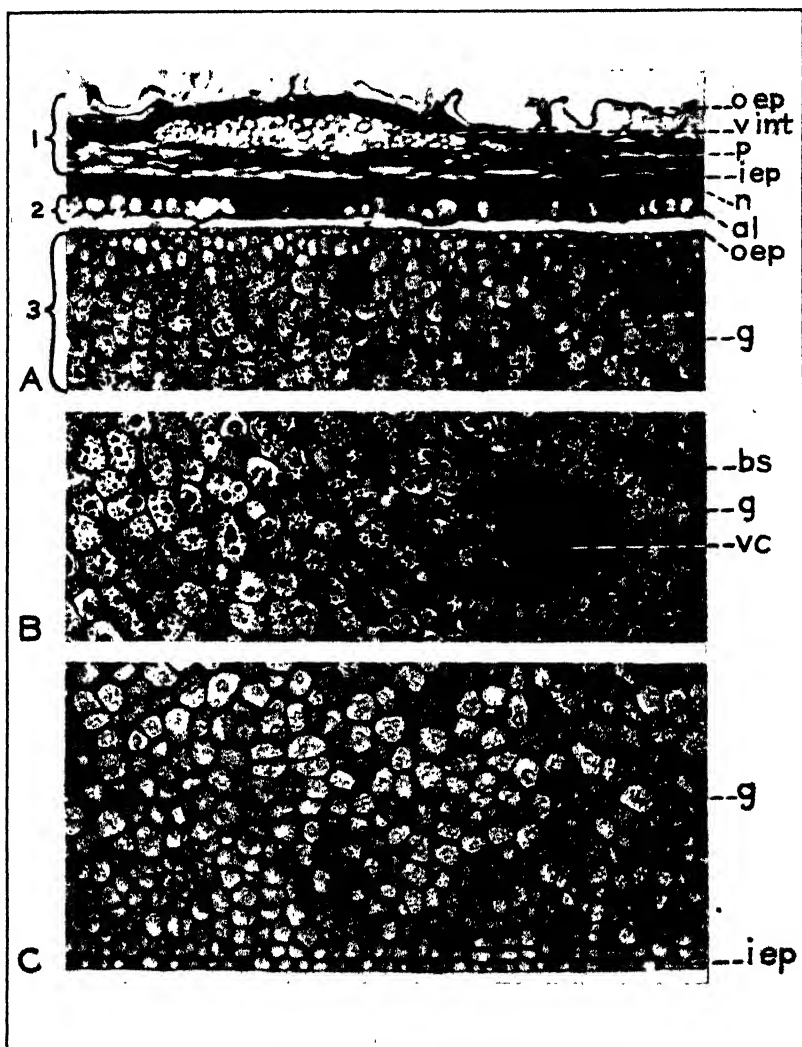


FIGURE 1.—Cross sections of Nonpareil almond. $\times 145$ A, Testa and outer portion of cotyledon. 1, Integuments: *o ep*, Outer epidermis; *p*, flattened cells of parenchyma; *v int*, vascular bundle; *i ep*, inner epidermis; 2, nucellus and endosperm; *n*, obliterated tissue of nucellus, *al*, aleurone layer; 3, cotyledon: *o ep*, outer, or lower, epidermis; *g*, ground tissue with proteing granules. B, Central portion of cotyledon: *g*, Ground tissue; *bs*, bundle sheath; *vc*, vascular bundle. C, Inner portion of cotyledon: *g*, Ground tissue; *i ep*, inner, or upper, epidermis.

in surface outline, usually somewhat elongated in the direction of the long axis of the seed, and without intercellular spaces. Each cell contains one or more large oil globules and numerous fine protein granules. The radicle is embedded in a mass of aleurone cells, but over the flat surface of the cotyledons the layer is usually one cell

thick, although it may be two or even three cells thick. The thickened areas may be due to tangential division of the cells, but in some instances they seem to be due to infoldings, the folds being parallel to the long axis of the seed. Individual aleurone cells vary in size and shape in different regions and in different varieties of nuts, but these differences are not significant in the determination of varieties. A thin layer of obliterated cells can sometimes be found between the two cotyledons or on the inner surface of the aleurone layer. This is composed of fragmentary traces of thin-walled endosperm tissue. It is especially evident in the chalazal region. Its presence between the cotyledons was first noted and figured by Hartwich (4).

The perisperm or nucellus (fig. 1, A, 2, *n*) consists of obliterated tissue, in which the individual cells are faintly visible after prolonged treatment with potassium hydroxide. They are very large, roughly 150 by 400 microns, with extremely delicate colorless walls. The outermost layer or epidermis of the nucellus consists of narrow, elongated cells with faintly beaded walls, regularly arranged with their long diameters parallel with the long axis of the seed. They are approximately 50 by 150 microns, although varying widely from these figures, which are the average of 125 measurements on four varieties, two domestic and two foreign. The variation in a single piece of tissue is often as great as in the different types of nuts.

The testa, or outer portion of the seed coat, is of integumentary origin. It is this part of the almond and related seeds that has received most attention from students of histology. Several points in structural anatomy have not yet been cleared up, and this still remains a fertile field for study.

The inner epidermis (fig. 1, A, 1, *i ep*) consists of small flat cells, roughly quadrilateral in surface view, with finely sinuate walls. These cells appear to be cutinized on their outer surface, the layer of cutin separating them from the extremely thin-walled epidermis of the nucellus. This interpretation is contrary to the opinion of Moeller and Thoms (8), who state that the cuticularized membrane belongs to the inner tissues which lie in contact with the brown integumentary layer, but is in agreement with that of Péchoutre (9), who states that the inner epidermis of the seed coat is slightly thickened and cutinized. The cell content forms a solid platelike mass, highly refractive, dark brown in color, and soluble in hot water and in 2 per cent potassium hydroxide, but insoluble in alcohol and ether.

The body of the testa is made up of flattened parenchyma cells (fig. 1, A, 1, *p*) separated into an inner and outer region. The inner region is made up of cells with colorless cellulose walls. The outer region consists of cells with lignified yellowish-brown walls. Between the two regions are the vascular bundles or veins (fig. 1, A, 1, *v int*) radiating from the chalaza and running practically parallel throughout the length of the seed to the micropylar tip. Along the veins are found rosette crystal aggregates of calcium oxalate, more abundant in hard-shelled types. The parenchyma cells of both the inner and outer regions when swelled in 2 per cent potassium hydroxide form a loose spongy tissue, the individual cells usually being in direct contact only by means of short blunt processes. Hence there is a large amount of intercellular space which is not apparent in the normal flattened condition of the cells in the ripened seed coat. The cells of the exterior subepidermal layers are much smaller, but are also

loosely arranged with much intercellular space. (Fig. 3, A, c.) The development of the integuments during the maturing of the seed has not been followed, and no statement appears in the literature consulted as to where the boundary between the two integuments lies. Péchoutre's sketches indicate that the tissues of the inner integument are more collapsed than those of the outer. It is natural to assume that the inner cells with cellulose walls are morphologically a part of the inner integument and the outer lignified cells are a part of the outer integument.

The outer epidermal layer of the testa, which forms the surface of the seed, has been subjected to profound modification. Most of the cells are enormously enlarged, and in many the walls are thickened and lignified. (Figs. 2, 3, and 4.⁶) The phloroglucinol-hydrochloric acid reaction shows the distribution and relative abundance of the lignified cells very clearly. (Fig. 4, B.) These lignified epidermal cells have attracted the attention of histologists for many years. They have been variously described as hair cells, barrel cells (Tonnenzellen), or balloon cells (Kugelnzellen), but are usually spoken of as stone cells. They are described by the early writers as egg-shaped or globular, though Young (13) figures extremely elongated cells, and Winton (11) states that they are commonly higher than broad and often elongated, like trichomes. In fact, there has been some discussion as to whether they are to be considered as epidermis or as trichomes.

The cell lumen is empty, but a thin protoplasmic layer is closely appressed to the inner surface of the cell wall. This is apparently what Tschirch (10) considers an inner layer of cell-wall substance, which he differentiates from the lignified portion of the wall. This layer gives a deep-blue reaction with ferric chloride, indicating the presence of tannin.

The epidermal cells vary noticeably in size, shape, degree of lignification, and distribution of lignified cells (figs. 2, 3, and 4), not only as between hard and soft shelled almonds, which has been noted by several writers, but also as between the varieties in either class, a fact which seems to have escaped the notice of earlier observers. In fact, Berg (1) states that there are no differences in structure between sweet and bitter almonds, the only difference between the two being in the cell contents of the kernels. Young (13) describes and figures the stone cells of the epidermis of the integuments of hard-shelled almonds, but says that in soft-shelled almonds a few of the epidermal cells have thicker walls than others, suggesting the stone cells found in the epidermis of the hard-shelled nut. Winton (11) agrees with Young. No varietal comparisons seem to have been made.

Wittmack and Buchwald (12) seem to have observed only the lignified cells and describe them as forming a discontinuous layer in the ripe seed. Hannig (3) first described the thin-walled nonlignified cells which are interspersed with the lignified cells, and remarked that they are sometimes found unaltered, but frequently are collapsed, and usually are completely crushed or altogether destroyed. This, he observed, gives the lignified cells the appearance of standing out from the surface singly or in groups; hence the opinion of earlier writers that they are hairs.

⁶ The writer is greatly indebted to G. L. Keenan, microanalyst, Food and Drug Administration, U. S. Department of Agriculture, for assistance in the preparation of these figures.

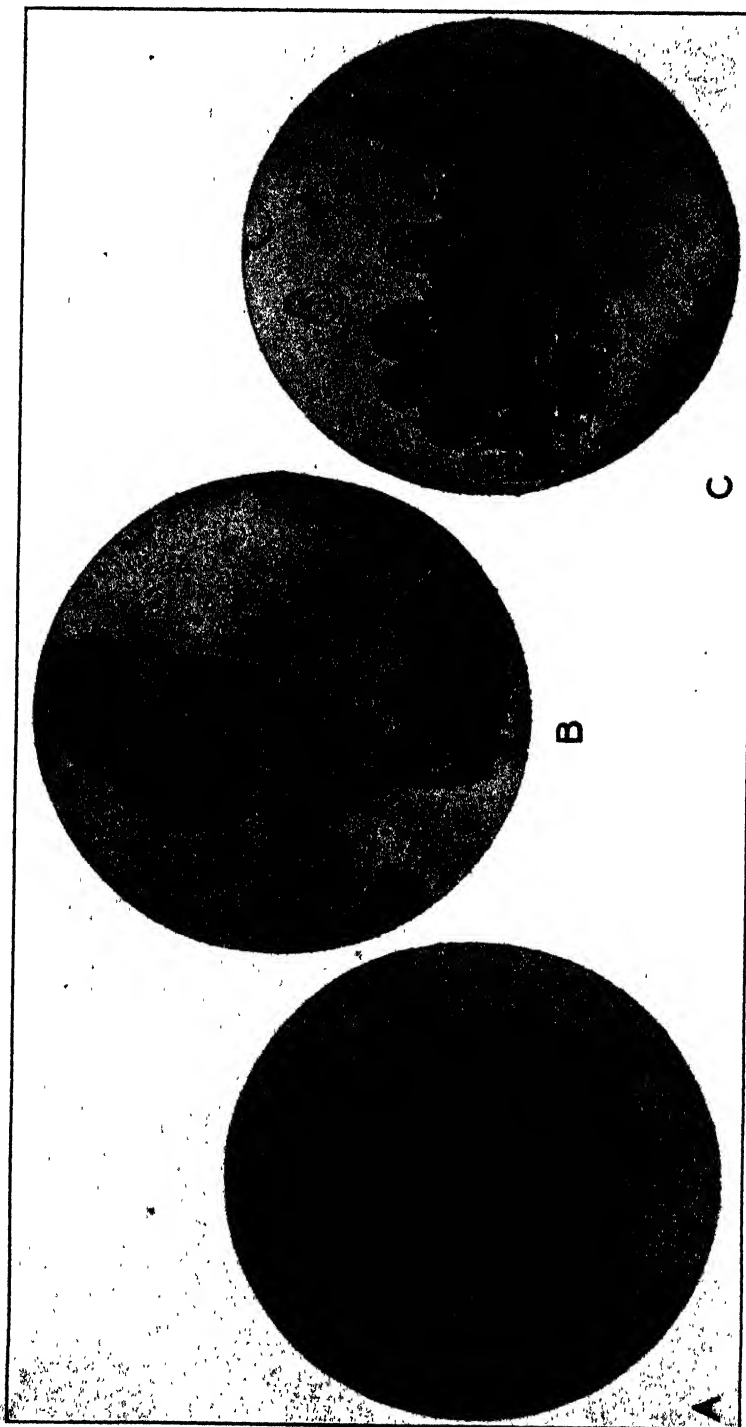


FIGURE 2.—A, Outer epidermis of testa of Nonpareil almond 1, Group of three stone cells from the side, showing heavy walls and pores; 2, surface view; a, Stone cells; b, thin-walled cells; B, Epidermal cells of Drake almond: a, Stone cells; b, thin-walled cells; c, subepidermal cells; C, Group of cells from Marcona almond; a, Stone cells; b, thin-walled cells; c, subepidermal cells. Macerated material, unstained. $\times 80$

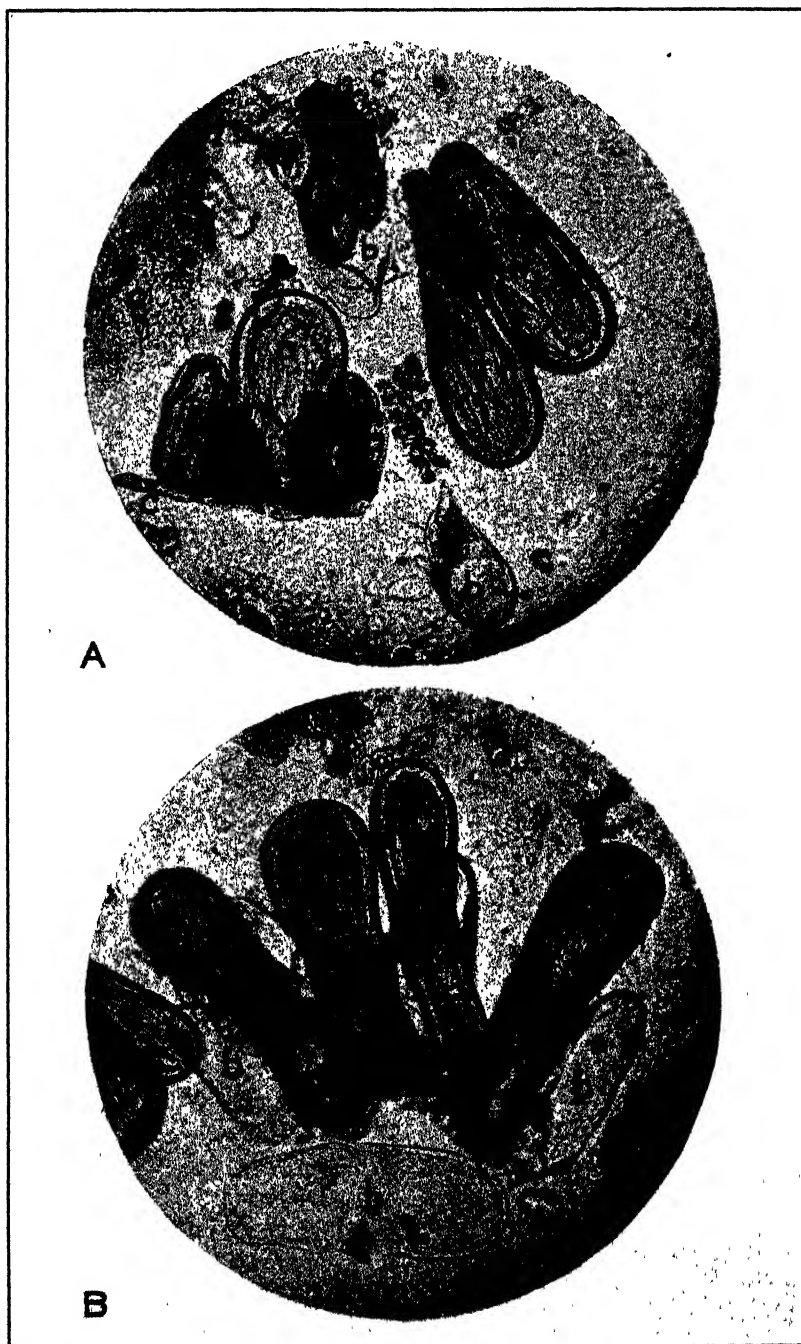


FIGURE 3.—Etna almond. A, Various types of cells from the testa: a, Stone cells of outer epidermis; b, thin-walled cells of outer epidermis; c, subepidermal cells. B, A group of very large epidermal cells: a, Stone cells; b, thin-walled cells. Macerated material, unstained. $\times 80$

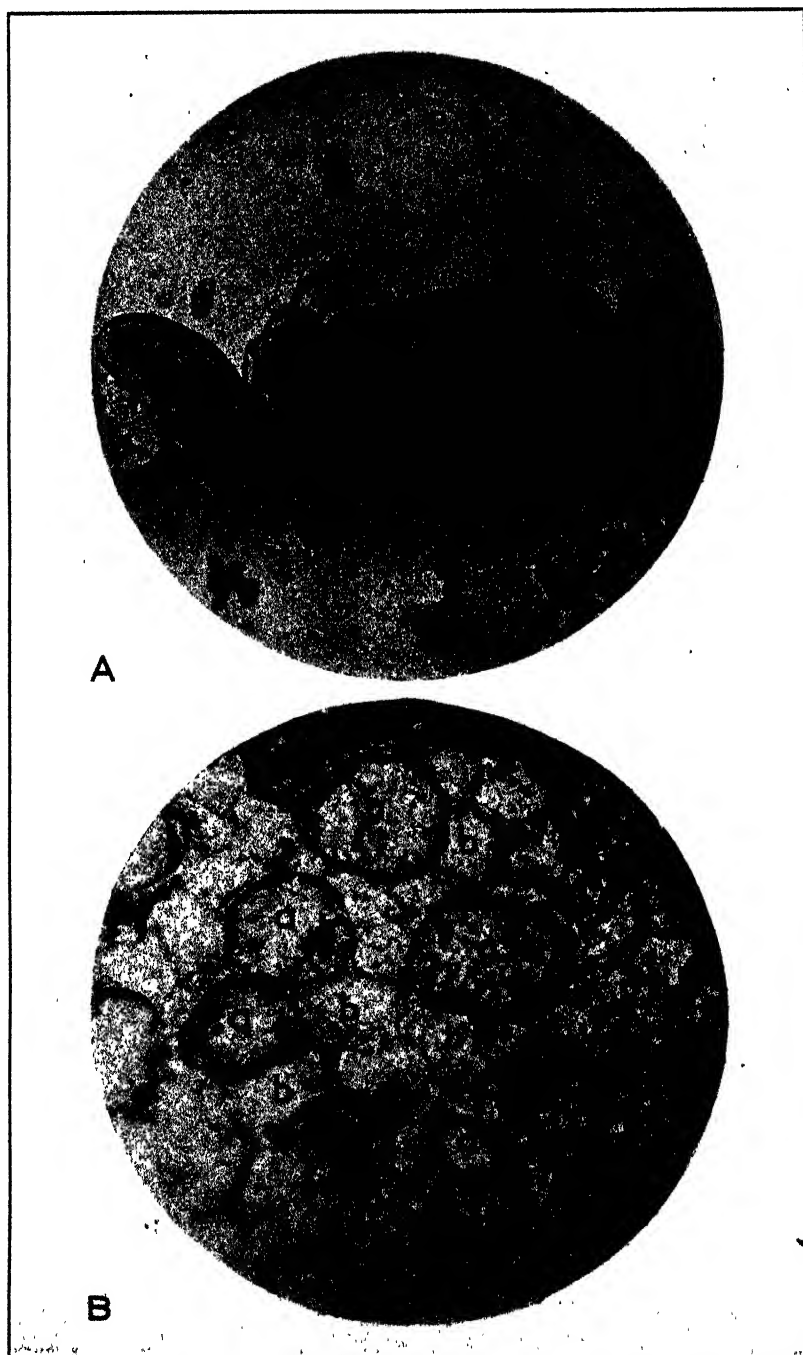


FIGURE 4.—Etna almond. A, Epidermal stone cells, over a vein, showing modified shapes. Macerated tissue, unstained. $\times 80$. B, Surface view of testa: a, Stone cell; b, thin-walled cell. Stained with phloroglucinol-hydrochloric acid. $\times 80$

The present study shows that in the varieties examined thick-walled lignified cells occur in the epidermal layer of the testa in both soft and hard shelled almonds. The lignified cells do not differ from the unlignified in size or shape, and are distributed irregularly over the surface, being apparently more closely crowded and more elongated over the radicle, and especially along the base of the raphe. Over the veins, and especially along the raphe, they often assume bizarre shapes as if stretched by the increasing growth of the seed. Over the chalaza the cells are much smaller.

The bases of the cells where in contact with each other and with the subepidermal layer are porous, the pores apparently connecting the cavities of adjacent cells. These pores are simple and unbranched, and usually round, but they may be elliptical, and several may be ranged at irregular intervals along shallow crescentic or serpentine grooves in the cell wall.

The free surface of the cell may be slightly domed or enormously extended, smooth on the outer surface and punctate or channeled on the inner. The elongated cells may be balloonlike, nipplelike, or beaked, and many of the individual cells are so large as to be plainly visible through a hand lens. Their rounded extremities give the roughened granular appearance to the shelled nut.

In the varieties of soft-shelled almonds studied the walls of the lignified cells are faintly colored a yellowish brown, and are thickened very little; the pores are comparatively few and aggregated in groups, and the inner surface of the outer wall is not punctate or ridged. (Fig. 2, A and B.) In all four varieties they give a very definite phloroglucinol-hydrochloric acid reaction. In the Nonpareil (fig. 2, A, 1), which appears smooth to the naked eye, the outer wall is only slightly domed, the depth of the cell being approximately equal to its diameter and averaging about 85 microns. In I. X. L. and Drake (fig. 2, B) the cells average approximately 130 microns in height and 100 microns in diameter, although cells were found more than 450 microns in height or diameter, such cells being easily visible with a hand lens. In Ne Plus Ultra the cells average approximately 175 microns in height and 135 microns in diameter, some cells measuring more than 450 microns.

In the hard-shelled almonds the lignified cells have heavily thickened walls, especially the free outer walls, which show stratification in all varieties when swelled in 2 per cent potassium hydroxide. They are strongly colored brown or yellowish brown. The Marcona (fig. 2, C) has the smallest cells measured, averaging approximately 130 microns in height and 75 microns in diameter, although reaching to from 300 to 370 microns in some instances. In the Etna (figs. 3 and 4) occurs the most marked elongation, the cells averaging approximately 300 microns in height and 130 microns in diameter, some cells being 650 microns in height, some 500 microns in width. In Valencia the cells average approximately 240 microns in height and 130 microns in diameter, with some cells exceeding 500 microns in either height or diameter. In Alicante the average measurements approximate 150 microns for height and 100 microns for diameter, although an occasional cell reaches 430 microns in height or 650 microns in diameter, the greatest diameter measured in any of the varieties.

In other words, the ratio of height to width in the four varieties of soft-shelled almonds averages 5:4, whereas in the four varieties of hard-shelled almonds the average ratio is 2:1. It must be borne in mind that extremes in height and diameter are not necessarily found in the same cell. Extremely elongated cells (fig. 3, B) may be found over any portion of the surface, with the exception of the chalaza, and they are especially abundant toward the apical end and along the margins of the raphe. Cells with the diameters stretched parallel to the long axis of the seed (fig. 4, A) are found immediately over the veins, and especially over the raphe. The averages given are based on at least 200 measurements for each variety.

A comparison of figures obtained in this study with those given by earlier workers is of interest. Such a comparison is presented in Tables 1 and 2.

TABLE 1.—Measurements of lignified cells of integuments of hard and soft shelled almonds

DOMESTIC ALMONDS, SOFT SHELLED						
Variety	Height (μ)			Diameter (μ)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Nonpareil.....	35	163	83	35	185	87
L. X. L.....	43	483	131	21	490	97
Drake.....	28	454	131	21	440	107
Ne Plus Ultra.....	21	483	177	28	461	136
Average.....			130			106

IMPORTED ALMONDS, HARD SHELLED						
Variety	Height (μ)			Diameter (μ)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Marccona.....	35	319	132	14	369	75
Etna.....	57	653	310	21	497	133
Valencia.....	35	547	241	21	516	132
Alicante.....	43	433	153	21	653	98
Average.....			209			109

TABLE 2.—Measurements of lignified cells of integuments of almonds

[Taken from the literature]

Author	Year	Variety	Height (μ)			Diameter (μ)		
			Minimum	Maximum	Average	Minimum	Maximum	Average
Wittmack & Buchwald (12).....	1901	Italian.....	90	109	175	60	84	100
J. Moeller (7).....	1905		136	159				
E. Hannig (8).....	1911		64	144				
A. Tschireh (10).....	1912	{Soft Hard Hard	120	(200) 335	100+	70	135 200	
W. J. Young (15).....	1912			400				
A. L. Winton (11).....	1916		60	400				
H. Kraemer (5).....	1928		70	175		65	100	

THE EMBRYO

In the dormant embryo of the ripened seed the massive cotyledons are usually flat and closely appressed, but an occasional seed is found in which one cotyledon is folded on itself longitudinally and the other is wrapped about it more or less completely. In most such cases the inner cotyledon is smaller, and it may be much underdeveloped or possibly even atrophied as a result of pressure from the surrounding cotyledon, which has a distinct advantage so far as expansion due to growth is concerned.

The cells of the ground tissue are fairly large and are packed with stored food material, each cell containing from one to several large protein granules and many small ones (fig. 1, A, B, and C, *g*), in addition to oil and soluble carbohydrates. In some cases there appear to be areas of physiological breakdown in otherwise normal cotyledons, the cells appearing empty and collapsed under the microscope.

The system of venation is well developed, forming a network which is very evident in longitudinal sections. The epidermal cells of the inner and outer surfaces of the cotyledon, corresponding to the upper and lower surfaces of a leaf, respectively, are small and narrow, the long axis of the cell being parallel with the long axis of the cotyledon. The outer or lower epidermal cells are much smaller than the inner and upper ones. Young's photomicrograph (2) is apparently a surface view of the outer epidermis. The difference in size of the two epidermal layers is well shown in Figure 1, A, 3, *o ep*, and C, *i ep*. This figure also shows the contrast in size between the cells of the epidermal layers and those of the ground tissue. The cell walls in all embryo cells are unmodified parenchyma and are extremely thin. Modifications extend only to size, shape, and content, with the exception of the beginning of thickening in the tracheal tissue of the veins.

SUMMARY AND CONCLUSIONS

A comparative study of the histology of four varieties of domestic soft-shelled almonds and four imported hard-shelled varieties is reported.

Evidence is presented to show that the chief difference between the two types of nuts lies in the structure of the cells of the outer epidermis of the testa, or the surface of the seed coat.

The modified epidermal cells of soft-shelled almonds are lignified, but only slightly as compared with those of hard-shelled almonds. They can therefore be considered as poorly developed stone cells.

Careful measurements indicate that in addition to being heavier walled the stone cells of the seed coat of hard-shelled almonds are more elongated than those of soft-shelled almonds, the ratio of length to width in hard-shelled almonds being approximately 2 to 1 and in soft-shelled almonds 5 to 4.

The importance of this study of the variation in the surface cells of the testa lies in the fact that with further examination and correlation of data it may be possible to develop an easily workable method for distinguishing the different varieties of almonds in the shelled condition, thus detecting or preventing misrepresentation or adulteration.

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THE ASPERGILLI AND THEIR RELATION TO DECAY IN APPLES¹

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INTRODUCTION

For the past three years investigations have been in progress at the Agricultural Experiment Station of the State College of Washington to determine the nature and identity of the fungi that cause decay of apples in cold storage. Many different organisms have been isolated and their pathogenicity determined by inoculation and reisolation. As a part of this work a study of the fungous flora of the normal apple has also been made.

WASHING EXPERIMENTS

In the investigation of the fungous flora of the normal apple the fruits used for washing were secured from the apple-growing districts of Wenatchee, Wash. Those used in 1927 were sorted and packed at Wenatchee and then shipped to Pullman, where they were placed in cold storage. In 1928, however, the apples were wrapped in sterile wraps as soon as they were picked in order to prevent spores from collecting on them while they were going through the processes of sorting and packing. They were then carefully packed and shipped to Pullman and placed in cold storage.

The apples were kept in cold storage until they were taken to the laboratory, where they were immediately washed. In order to prevent contamination in transferring the fruit from storage to the laboratory, each apple was handled in such a way as not to disturb the wraps.

The walls and working table of the culture room were washed with HgCl_2 , 1-500, and the air was thoroughly atomized with a similar solution before the washing of each set of apples.

Each apple was carefully unwrapped and placed in a sterile damp chamber which contained 100 c. c. of sterile water. The lid of the damp chamber was raised only high enough to permit the handling of the apple. The fruit was held firmly with sterile forceps and thoroughly scrubbed with a sterile, $\frac{1}{2}$ -inch, stiff stencil brush for five minutes. Plates were made in triplicate with one-eighth, one-fourth, and one-half cubic centimeter, respectively, from the thoroughly agitated wash water by removing the suspension to sterile Petri dishes with 1 c. c. sterile pipettes, and then pouring the medium. The medium used was potato agar containing 2 per cent dextrose. To inhibit bacterial growth 1 drop of 25 per cent lactic acid was added to each tube of medium before it was poured. The suspension and medium were

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thoroughly mixed by a circular motion and the plates allowed to harden. They were then incubated in the laboratory at ordinary room temperature, and after three days the number and the type of colonies were recorded. Isolations were made from the various colonies, and stock cultures of each were stored in the low-temperature chest for later identification.

After several sets of apples had been washed, it was decided to find how many spores remained on the fruit after washing. Sartory and Fillassier³ had found that a large number of spores remained on the surface of fruit after the first washing, but that the number was greatly reduced by a second washing and still further reduced by a third.

After an apple had been thoroughly washed for five minutes, as described above, it was rinsed in sterile water and thoroughly washed a second time for five minutes in 100 c. c. sterile water as before. It was again rinsed in sterile water and washed a third time. The waters from the first, second, and third washings were plated out by the method described above and a count of the number of colonies produced from each washing was taken after three days' incubation at room temperature. A number of apples (125 size) were tested in this way. The results of these tests are given in Table 1.

The results here shown are very similar to those obtained by Sartory and Fillassier. All the spores were not removed by the first washing, but the number was greatly reduced by a second and a third washing.

TABLE 1.—*Number of spores (colony counts) per apple obtained in successive washings of normal sound fruit*

[Apples 1-6, wrapped in orchard immediately after picking, apples 7-9, storage apples]

Apple No	Number of spores in—			Total number of spores	Apple No.	Number of spores in—			Total number of spores
	First washing	Second washing	Third washing			First washing	Second washing	Third washing	
1.....	108,300	29,000	13,400	150,700	6.....	11,100	2,200	700	14,000
2.....	31,800	1,300	1,000	34,100	7.....	138,400	82,700	13,200	234,300
3.....	55,600	44,600	12,400	112,600	8.....	146,700	82,000	42,900	271,600
4.....	60,300	37,300	14,400	112,000	9.....	108,000	47,800	9,200	165,000
5.....	108,000	32,000	19,100	159,100					

DESCRIPTION OF FORMS

Thom and Church⁴ have made a careful study of the *Aspergilli*, and their nomenclature is followed in this paper.

The cultures on which the following descriptions were based were incubated in the laboratory at room temperature and observations were made throughout the entire growth of the colonies. Single-spore cultures growing on Czapeks' solution agar⁵ as described by Thom and Church were used in all cases. Some of the forms have been definitely assigned to a given species, while the identification of other forms is so uncertain that they have been placed in the nearest form groups.

³ SARTORY, A., and FILLASSIER, A. LES FRUITS PORTEURS DE MICROBES. Compt. Rend. Soc. Biol. [Paris] (Année 61, t. 2) 67: 445-447. 1909.

⁴ THOM, C., and CHURCH, M. B. THE ASPERGILLI. 272 p., illus. Baltimore. 1926.

⁵ Czapeks' solution agar: Water 1,000 c. c.; sodium nitrate 3 gm.; potassium phosphate (K_2HPO_4) 1 gm.; magnesium sulphate 0.5 gm.; potassium chloride 0.5 gm.; ferrous sulphate 0.01 gm.; sucrose 30 gm.; agar-agar 15 gm.

All the forms were washed from normal Jonathan apples, Nos. 1 to 5, from the 1927 crop and Nos. 6 to 11 from the 1928 crop.

FORM NO. 1

Form 1 (fig. 1) belongs to the *Aspergillus ochraceus* group, *sulphureus* series, but does not agree in all respects with any of the species described by Thom and Church.

Colonies pale yellow, close to Naples yellow (Ridgway, Pl. XVI),⁶ growth regular; reverse at first white with yellowing center, becoming reddish brown in old cultures. Conidial heads globose, up to 200μ in diameter, mostly 160μ . Stalks rising from submerged hyphae, up to $1,500\mu$ in length and up to 16μ in diameter, walls light yellow, pitted; vesicle globose, up to 60μ in diameter; sterigmata in two series, primary sterigmata about twice as long as the secondary and closely

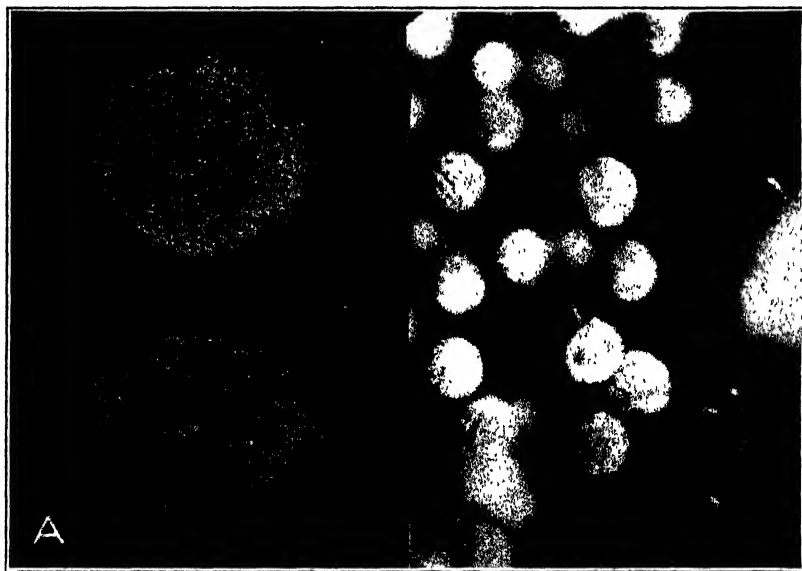


FIGURE 1. - *Aspergillus* form No. 1. Plate culture on Czapek's solution agar incubated at 20°C . for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

packed to form a radiate head; conidia globose to subglobose, 2μ to 3.5μ in diameter. Perithecia not found, sclerotia purple, globose, up to 800μ in diameter, single, scattered over entire colony.

FORM NO. 2

Form 2 (fig. 2) is easily placed in the *Aspergillus tamarii* group, and is practically identical with *A. tamarii*.

The vegetative hyphae are mostly aerial, white, and more or less cottony. The fruiting areas are pale yellow at first, soon becoming a deep yellow, then passing through the orange-yellows to a dark brown in old cultures; reverse cream colored at first, becoming light brown with age. Conidial heads more or less globose when young, soon becoming very loose, the conidial chains diverging in all directions, up to 500μ in diameter with radiating chains or columns of conidia. Stalks apparently smooth, slightly pitted in some cases, up to $1,500\mu$ in length and up to 16μ in diameter; vesicles globose, up to 75μ in diameter; sterigmata one series, up to 40μ long in larger heads and up to 4μ wide; conidia globose to pyriform, tuberculate, 4μ to 6μ in diameter. Perithecia none. Sclerotia none.

⁶ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

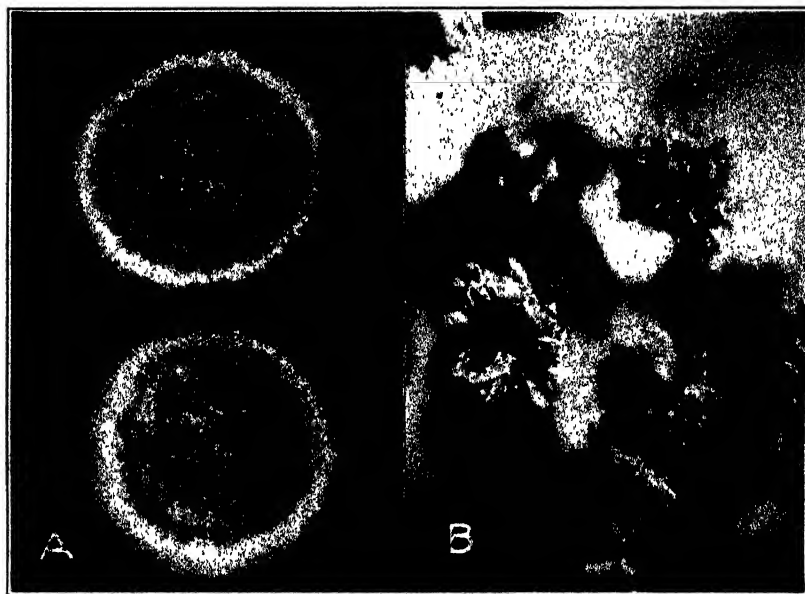


FIGURE 2.—*Aspergillus* form No. 2. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conical heads (B). $\times 50$

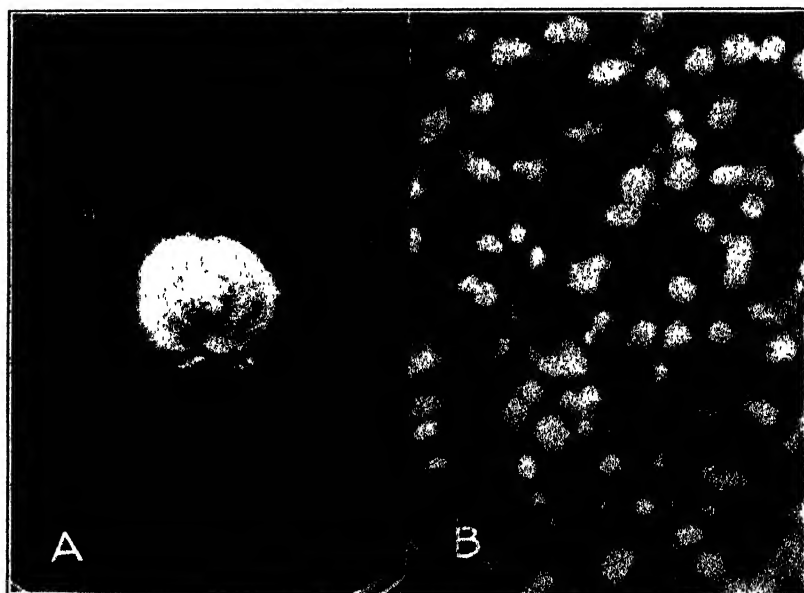


FIGURE 3.—*Aspergillus* form No. 3. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conical heads (B). $\times 50$

FORM NO. 3

Form 3 (fig. 3) is placed in the white-spored *Aspergillus* group, but further identification has not been made.

Colony white at first, turning to a pale pink with a tinge of yellow around the edge in old cultures; reverse yellow to greenish orange. Conidial heads varying greatly in size and shape in the same colony, from hemispherical to columnar, stalks smooth, up to 250μ in length and up to 6μ in width, colorless, sparingly septate; vesicles more or less flask-shaped, fertile on upper half only, up to 16μ in diameter; conidia globose, 1.5μ by 2.5μ in diameter, smooth; sterigmata in two series, primary 8μ to 12μ by 3μ to 5μ ; secondary 4μ to 6μ by 2μ to 3μ , compact. Perithecia none. Sclerotia none.

FORM NO. 4

Form 4 (fig. 4) is very closely related to *Aspergillus ustus*.

Colonies fluffy or more or less cottony at first, turning brown in the center and spreading toward the edge as they grow older; reverse pale yellow at first, becom-

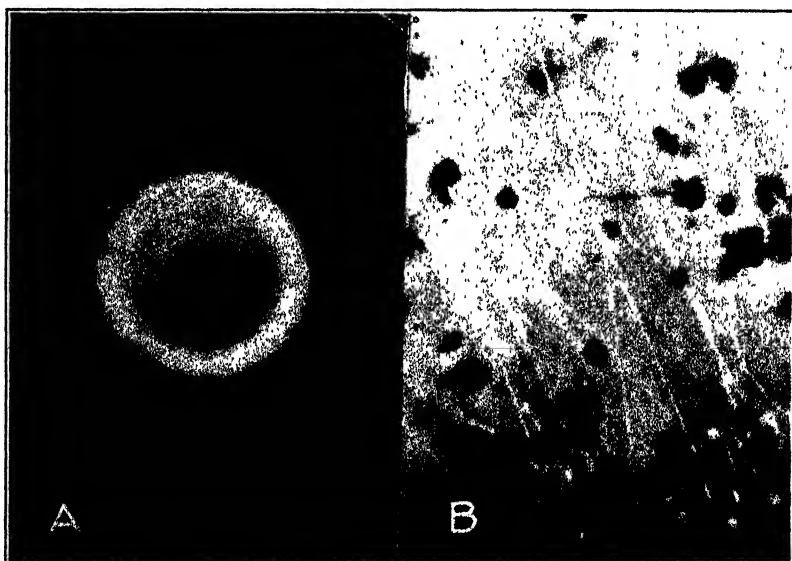


FIGURE 4.—*Aspergillus* form No. 4. Plate culture on Czapek's solution agar incubated at 20°C . for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

ing deeper yellow to brownish yellow with age. Conidial heads loose, irregular, hemispherical, up to 100μ in diameter. Stalks smooth, colored, rising from aerial hyphae, up to 300μ by 5μ to 8μ . Vesicles developing from slight swellings of the stalk, through flask-shaped to more or less globose, up to 16μ in diameter. Sterigmata in two series, semiradiate. Conidia globose, 3.5μ to 4.5μ , colored, spinulose. Perithecia none. Sclerotia none.

FORM NO. 5

Form 5 (fig. 5) belongs to the *Aspergillus niger* group, and because of the large variation within the species, may easily be classed as *A. niger* Van Tieg.

Colonies blackish brown to black; reverse white, wrinkled. Conidial heads globose at first, up to 300μ in diameter, usually splitting with age in two or more ways, forming radiating conidial columns; stalks rising from both submerged and aerial hyphae, up to 2.5 mm. in length and up to 20μ in width, walls yellowish, 3.5μ to 4μ thick, smooth, nonseptate; vesicles globose, up to 60μ in diameter; conidia globose, 3.5μ to 4μ in diameter, colored, spinulose. Sterigmata in one and two series, primary sterigmata up to 60μ in length and up to 4μ in width, secondary sterigmata up to 8μ by 2μ or 3μ . Perithecia none. Sclerotia none.

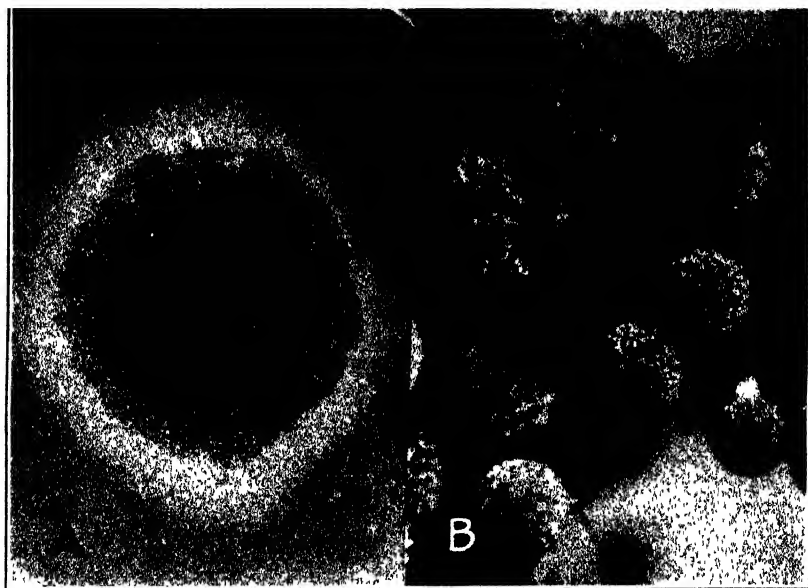


FIGURE 5.—*Aspergillus* form No. 5. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

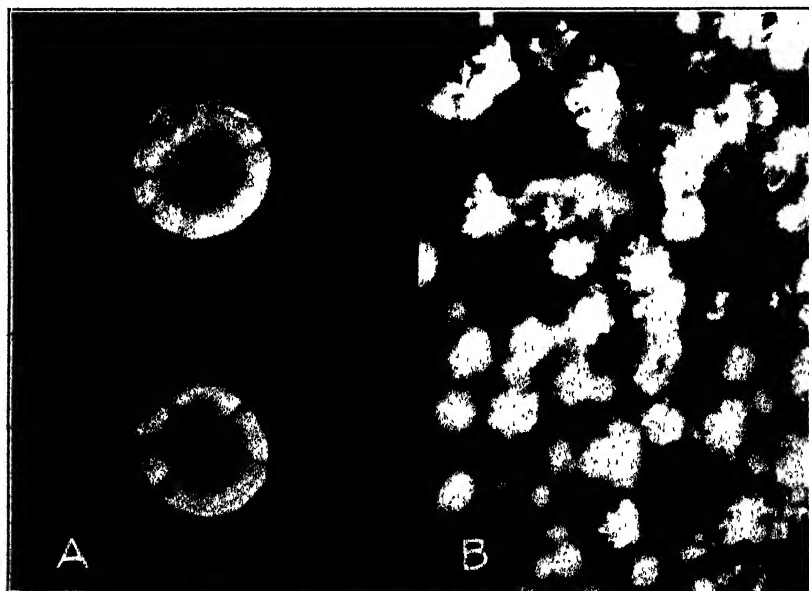


FIGURE 6.—*Aspergillus* form No. 6. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

FORM NO. 6

Form 6 (fig. 6) is very closely related to *Aspergillus sydowi* (Bainier and Sartory) T. and C., and is perhaps identical with it.⁷

Colonies blue-green, sometimes with age turning a more or less rusty color in the denser portions; surface convoluted; reverse at first orange, becoming deep red with age. Conidial heads loosely globose or radiate, up to 160μ in diameter. Stalks rising from more or less submerged hyphae except in center of colony, where they rise from aerial hyphae, up to 500μ by 8μ , colorless, smooth, septate; vesicles globose to flask-shaped, up to 20μ in diameter; sterigmata radiate in two series, primary 4μ to 8μ by 2μ to 3μ , secondary 4μ by 2μ ; sometimes in one series, 6μ to 10μ by 2μ to 4μ . Conidia globose, 2μ to 3μ in diameter, spinulose. Perithecia none. Sclerotia none.

FORM NO. 7

Form 7 (fig. 7) is perhaps identical with *Aspergillus terreus* Thom as described by Thom and Church.

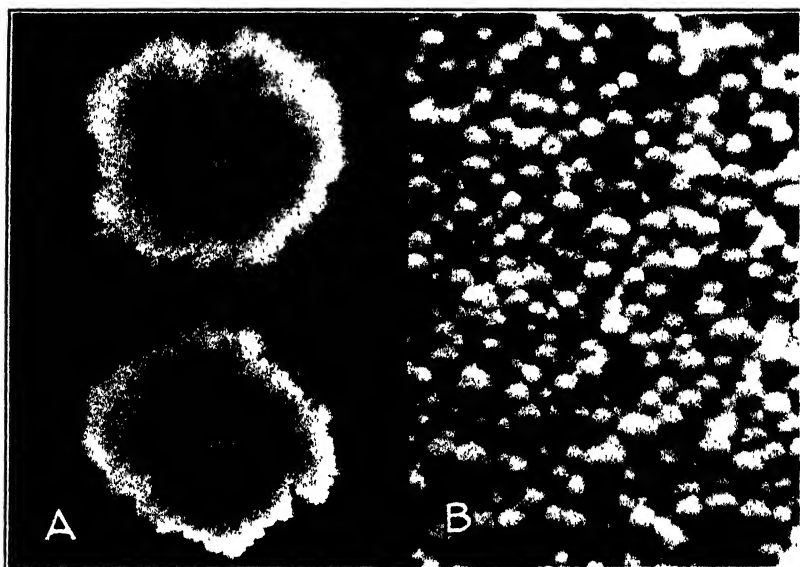


FIGURE 7.—*Aspergillus* form No. 7. Plate culture on Czapek's solution agar incubated at 20°C . for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

Colonies light pink around the edges, deepening toward the center becoming cinnamon-rufous brown (Ridgway,⁸ Pl. XIV), in old cultures. Conidial heads columnar, up to 300μ long and up to 85μ wide, densely crowded. Stalks short, up to 130μ long and up to 8μ wide, rising from submerged hyphae, more or less flexuous, smooth, densely crowded. Vesicles more or less flask shaped, up to 18μ in diameter. Sterigmata in one or two series. Conidia globose, 2μ to 3μ , smooth. Perithecia none. Sclerotia none.

FORM NO. 8

Form 8 (fig. 8) belongs to the *Aspergillus fumigatus* group. Identification was not carried farther because of the lack of perithecial production upon which further division might have been made (Thom and Church⁹).

Colonies bluish green at first, turning to dark green with age; reverse yellowish green. Conidial heads columnar, compact, up to 300μ long, mostly about 200μ long, and up to 68μ wide. Stalks rising from submerged hyphae, smooth, slightly colored, nonseptate, up to 500μ long and up to 12μ wide; vesicles flask-

⁷THOM, C., and CHURCH, M. B. Op. cit., p. 147-148.

⁸RIDGWAY, R. Op. cit.

⁹THOM, C., and CHURCH, M. B. Op. cit.

shaped, up to 26μ in diameter, fertile only on upper half; sterigmata in one series, 6μ to 8μ by 2μ to 3.5μ , closely packed; conidia globose, 2μ to 4μ in diameter spinulose. Perithecia none. Sclerotia none.

FORM NO. 9

Form 9 (fig. 9) may be referred very definitely to the *Aspergillus glaucus* group.

Colonies light gray-green near edge, deepening toward the center (glaucous-green, Ridgway, P. XXXIII);¹⁰ reverse cream colored; growth regular. Conidial heads loosely columnar, up to 250μ in length, width varying greatly due to looseness of head, averaging 50μ ; conidial chains spreading and more or less tangled. Stalks rising from submerged hyphae, varying from a few to 160μ long by 6μ wide, smooth, septate, sometimes swelling toward the vesicle, and in a few cases, branching near the tip, thus producing two heads. Vesicles ranging in shape from slightly apical swellings of the stalks or flask-shaped to more or less globose;

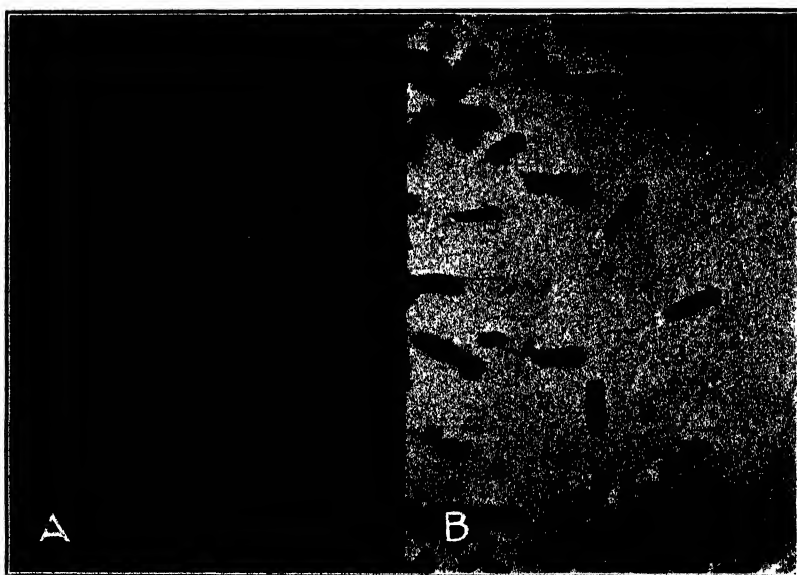


FIGURE 8.—*Aspergillus* form No. 8. Plate culture on Czapek's solution agar incubated at 20°C . for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

up to 16μ in diameter, in many cases proliferating to form short secondary stalks with small heads. Sterigmata one series, short. Conidia globose, up to 4μ in diameter, smooth. Perithecia none. Sclerotia none. Chlamydospores numerous.

FORM NO. 10

Form 10 (fig. 10) belongs to the *Aspergillus nidulans* group, and is perhaps identical with *A. nidulans*.

Colonies green, edges irregular, yellow perithecia soon developing singly, scattered over entire colony; reverse light brown at first, turning reddish brown. Conidial heads columnar, compact, up to 300μ long and up to 60μ wide; stalks smooth, more or less flexuose, branched in some cases, yellowish brown, septate, up to 100μ long and up to 8μ wide; vesicles domelike swellings at end of conidiophores, up to 15μ in diameter; sterigmata in two series, primary up to 8μ by 2μ to 3μ , secondary up to 5μ by 2μ . Conidia globose 3.5μ to 4.5μ in diameter, spinulose. Perithecia up to 650μ in diameter including the Hülle cells, almost black; asci numerous, globose, 8 spored, 10μ to 12μ in diameter; ascospores globose to elliptical 3.5μ to 4.5μ . Sclerotia none.

¹⁰ RIDGWAY, R. Op. cit.]

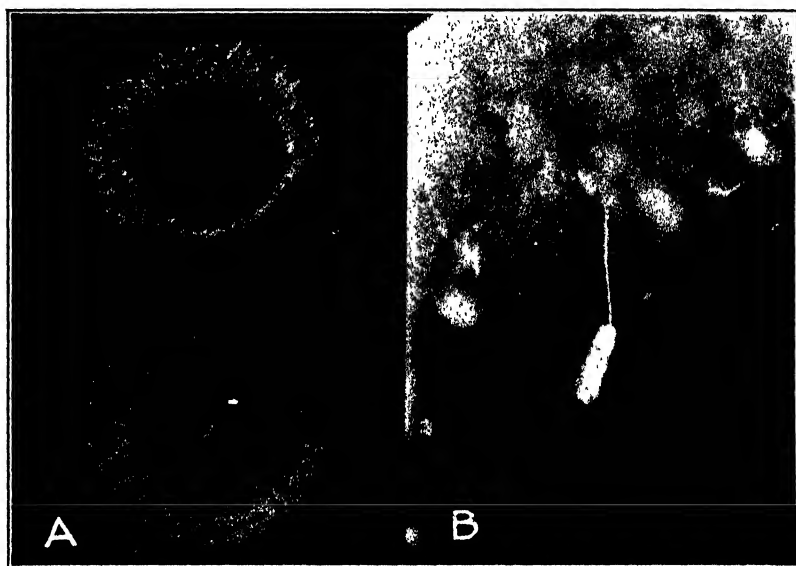


FIGURE 9.—*Aspergillus* form No. 9. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$



FIGURE 10.—*Aspergillus* form No. 10. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

FORM NO. 11

Form 11 (fig. 11) resembles *Aspergillus sydowi* (Bainier and Sartory) T. and C. in colony characteristics but differs somewhat in other respects.

Colonies blue green, tending toward green with age; reverse light brown with brown center, becoming reddish brown with age. Conidial heads varying greatly, loosely hemispherical with radiating chains to very loosely columnar. Resembles *Aspergillus sydowi* as described by Thom and Church¹¹ in reduced conidial apparatus frequent as small heads, pectinate clusters of branched sterigmata or short conidiophores or single branched sterigmata sessile on trailing hyphae. Stalks smooth, sparsely septate, rising mostly from submerged hyphae, up to 500μ by up to 6μ . Vesicles more or less flask shaped, upper half fertile, up to 15μ in diameter. Sterigmata in two series, primary and secondary nearly equal

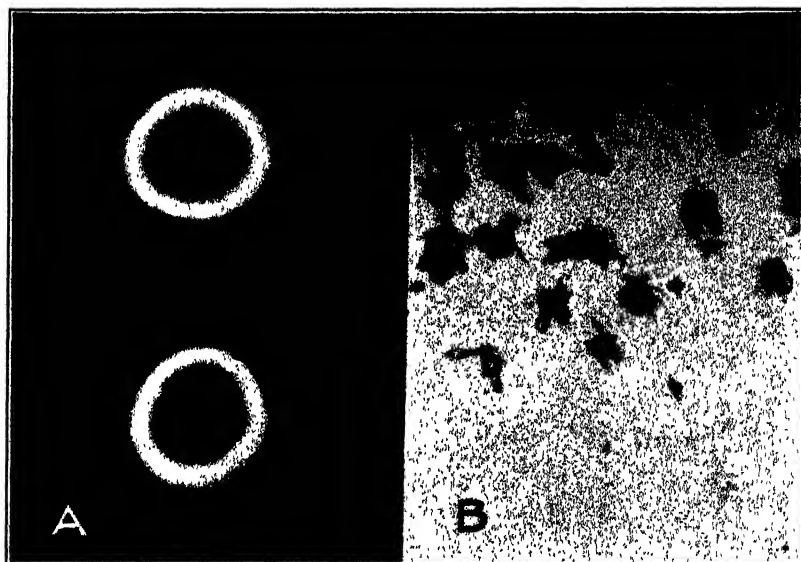


FIGURE 11.—*Aspergillus* form No. 11. Plate culture on Czapek's solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). $\times 50$

in size, up to 18μ by up to 3μ . Conidia globose, 3μ to 4μ in diameter, spinulose. Perithecia none. Sclerotia none.

INOCULATION EXPERIMENTS

To determine the pathogenicity of the various forms of *Aspergilli* described above a number of inoculation experiments were made. In these experiments Jonathan apples were used. The apples were packed in Wenatchee and shipped to Pullman, where they were kept in storage until used. All the apples were from the same lot and were as nearly alike in size and degree of maturity as could be secured.

Brooks and Cooley¹² state that the rapidity of rotting depends greatly upon the variety and maturity of the fruit. The writer has also found this to be the case, particularly with the weaker organisms.

Inoculations from single-spore cultures of the forms described above were made according to a method worked out by the author.¹³ The

¹¹ THOM, C., and CHURCH, M. B. Op. cit., p. 148.

¹² BROOKS, C., and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. Jour. Agr. Research 8: 139-164, illus. 1917.

¹³ HUBER, G. A. A SIMPLE METHOD OF INOCULATING THE APPLE. Phytopathology 20: 101-102. 1930.

inoculated fruits were placed under various conditions of storage, and subsequent reisolations were made of the forms causing decay to establish their pathogenicity.

Six apples were inoculated. Three inoculations were made in each with each of the 11 forms of *Aspergillus* and the fruits were placed in each of the 11 forms of *Aspergillus* and the fruits were placed in the cold-storage room, where the temperature was maintained at about 0° C. A second lot of apples, inoculated as above, was placed in the common storage room in which the temperature ranged from 10° to 12°; a third lot was placed in the plant pathology storeroom, in which the temperature ranged from 18° to 22° C. The rate of decay caused by the different forms at temperatures of 10° to 12° and 18° to 22° is given in Table 2. The results here presented show that seven of the forms are capable, under favorable storage conditions, of causing decay when inoculated into sound apples. The pathogenicity of some of the forms is very weak, even under the most favorable storage conditions, while others develop rapid decay. That the rate of decay varies greatly in the different forms is seen in Figures 12 and 13.

Under cold-storage conditions (about 0° C.) none of the forms of *Aspergillus* had caused decay by the end of 12 weeks, when the final examination was made. It is a well-known fact that species of *Aspergillus* are relatively high-temperature organisms; therefore it was not expected that decay would develop at such low temperatures. As a matter of fact, in the three years during which storage-rot investigations have been in progress at this station, no form of *Aspergillus* has been cultured from more than a thousand isolations made from rots of cold-storage apples. Apples were inoculated with *Aspergillus niger* (form 5), which is the most rapid rot-producing form of *Aspergillus*, and incubated at 5° in the ice chest. At this temperature also the organism failed to produce decay at the end of 12 weeks.

TABLE 2.—Rate of decay caused by forms of *Aspergillus* in apples in common storage and storeroom temperatures

Aspergillus form No.	Rate of decay at a temperature of 10° to 12° C.		Rate of decay at a temperature of 18° to 22° C.		Aspergillus form No.	Rate of decay at a temperature of 10° to 12° C.		Rate of decay at a temperature of 18° to 22° C.	
	Average decay per week	Total decay in 12 weeks	Average decay per week	Total decay in 4 weeks		Average decay per week	Total decay in 12 weeks	Average decay per week	Total decay in 4 weeks
1.....	(1)	(1)	0.9	3.6	7.....	0.083	1	0.925	3.7
2.....	¹ 0.075	0.9	.35	1.4	8.....	(1)	(1)	(1)	(1)
3.....	(1)	(1)	.35	1.4	9.....	?	(1)	.4	1.6
4.....	(1)	(1)	(1)	(1)	10.....	?	(1)	.45	1.8
5.....	.258	3.1	2.8	(1)	11.....	?	(1)	(1)	(1)
6.....	(1)	(1)	(1)	(1)					

¹ No decay.

² The figures represent the surface diameter of the rotted areas in centimeters.

³ Entire apple decayed.

⁴ Decay starting.

DESCRIPTION OF DECAY CAUSED BY FORMS OF ASPERGILLUS

The following descriptions give the character and type of decay produced by the pathogenic forms. These were taken from decayed areas resulting from inoculations made January 18, 1929, and incubated for one month at storeroom temperature ranging from 18° to 22° C.

FORM No. 1.—The exterior of the decayed portion is a deep brown in color, showing a tendency to form concentric rings which in some cases become quite pronounced. (Fig. 13, A.) The decayed flesh is brown, firm, and moist, although it appears somewhat dry. The decayed area generally has the form of a slightly flattened cone, the diameter at the surface of the fruit exceeding the depth to which it penetrates. (Fig. 14, A.)

FORM No. 2.—The surface of the decayed area is somewhat sunken, with two distinct zones. On light-colored apples, the inner zone is

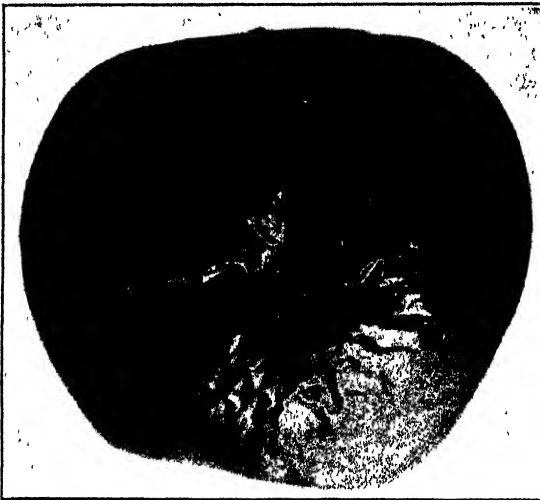


FIGURE 12.—External view of decay in Jonathan apple showing concentric rings caused by *Aspergillus* form 5

more or less reddish brown, while the outer zone is usually a light brown. On darker colored apples the outer zone becomes darker, and is a dark red in highly colored apples in contrast to the reddish brown inner zone. (Fig. 13, B.) The decayed flesh is brown, very dry, and corky in appearance. The tissue is tough and has a somewhat bitter taste. The organism advances into the tissue very irregularly; however, the decayed area has a more or less conical appearance. (Fig. 14, B.)

FORM No. 3.—This form produces a very peculiar type of rot. There is no sign of decay on the surface of the apple until the rot has advanced considerably within the flesh. At first it has the appearance of a water-soaked area, without definite shape. Later as the infected area enlarges it becomes light brown in color, although the edges still retain the water-soaked appearance and very irregular outline. (Fig. 13, C.) The affected tissue is light brown in color, firm, more or less moist, and subglobose in shape. The infection takes place at the bottom of the puncture and spreads more rapidly toward the interior than toward the exterior of the apple. (Fig. 15, A.)

It was difficult to determine the rate at which the decay advanced with this form because of the peculiar type of development. Table 2 shows the measurement of the decay when the final record was taken.

FORM No. 5.—This form causes a much more rapid decay than any of the others. The exterior of the decayed area shows a distinct

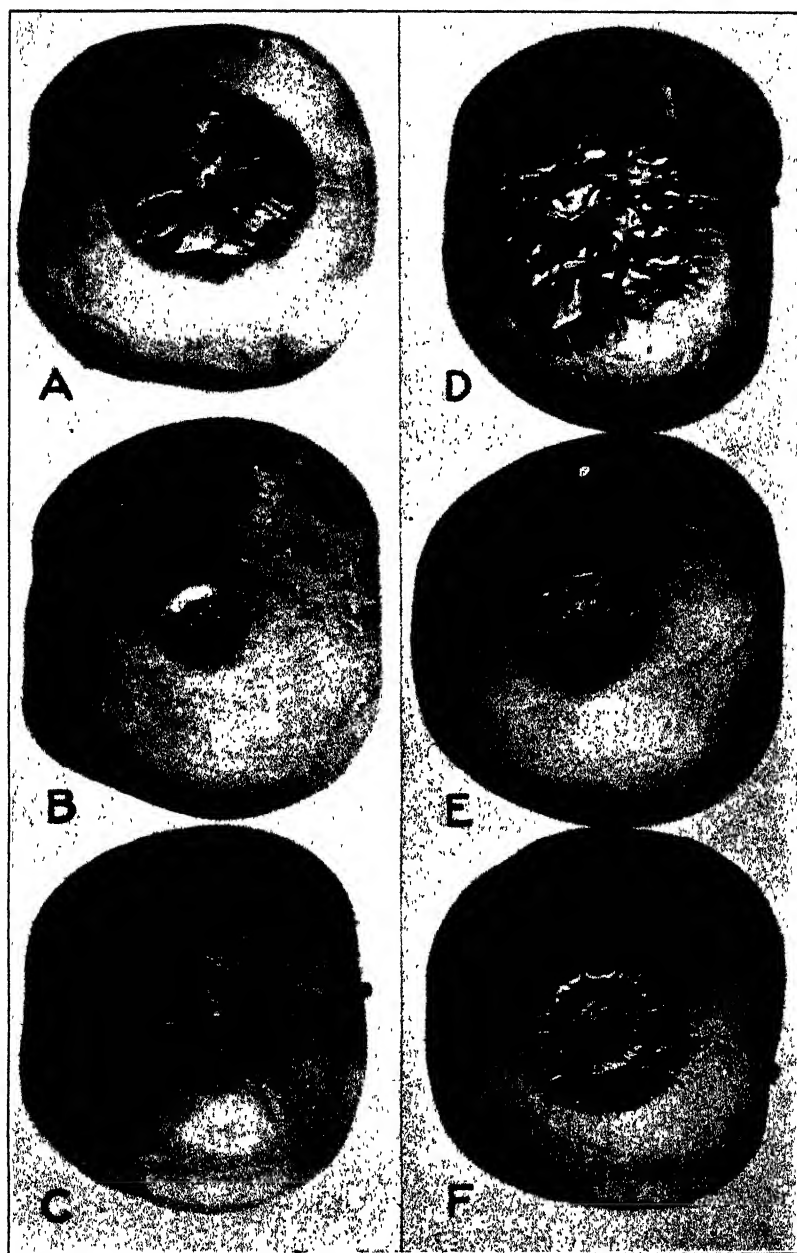


FIGURE 13.—Jonathan apples showing external view of decays caused by forms of *Aspergillus*. Decay caused by *Aspergillus* form No. 1, (A); No. 2, (B); No. 3, (C); No. 7, (D); No. 9, (E); No. 10 (F)

zonation in a large majority of cases. (Fig. 12.) As the decay advances, the original color of the apple disappears and the decayed area becomes very light in color with a pinkish cast. The pinkish coloration is much deeper in highly colored apples than in those with little color. The decayed tissue is extremely soft and watery, but there is no sign of the fungus on the unbroken epidermis over the decayed area. The decayed area is conical in shape, the point of the cone soon extending to the core of the apple. (Fig. 15, B.)

FORM No. 7.—The decay caused by this form is very similar to that caused by form 1. The exterior is brown with a slight tinge of green, but it does not show the concentric rings that generally appear in form 1. (Fig. 13, D.) The decayed flesh is also brown with a light greenish cast. It is conical in shape, extending much deeper

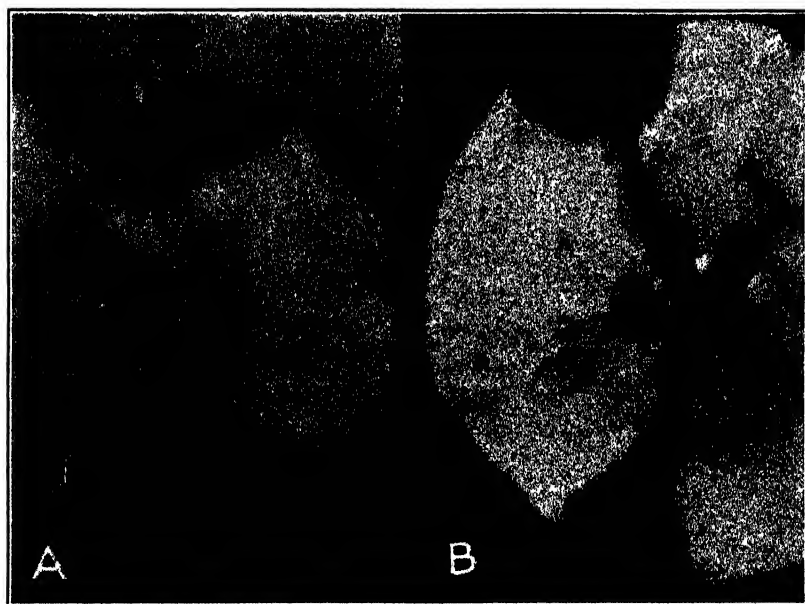


FIGURE 14.—Cross section of Jonathan apple showing type of decay caused by *Aspargillus* forms No. 1, (A), and No. 2, (B)

into the fruit in proportion to the diameter at the surface than the decay caused by form 1. (Fig. 16, A.)

FORM No. 9.—The surface of the decay caused by this form is greenish brown. The margin of the decay fuses more or less with the healthy portion, giving no distinct line of separation between the two. (Fig. 13, E.) The decayed flesh is brown, showing a greenish tinge. It is more or less dry and spongy. The decayed area is somewhat conical in shape, tending toward subglobose in many cases. (Fig. 16, B.)

FORM No. 10.—The exterior of the decayed portion is brown in color, with a very distinct line of demarcation between healthy and decayed areas. (Fig. 13, F.) The decayed flesh is brown, dry, corky, and irregular in shape. (Fig. 17, A.)

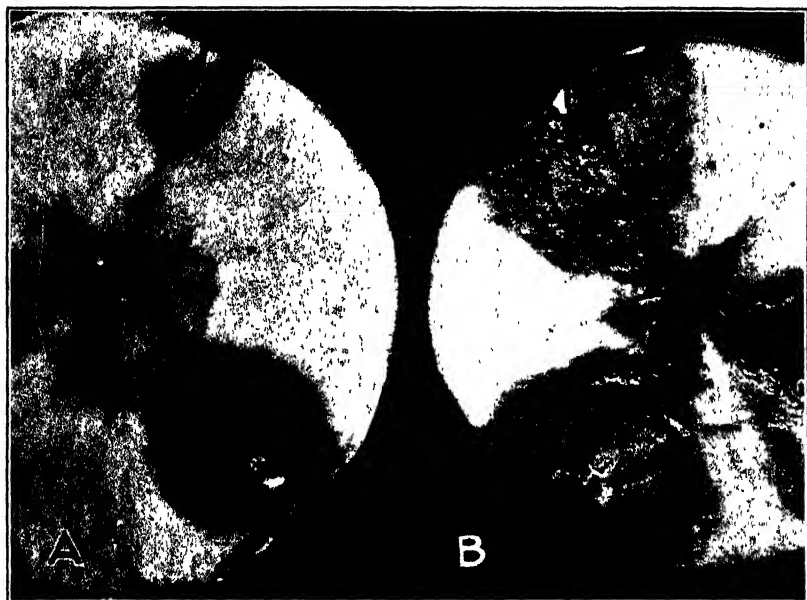


FIGURE 15 - Cross section of Jonathan apple showing type of decay caused by *Aspergillus* forms No 3 (A) and No 5 (B)

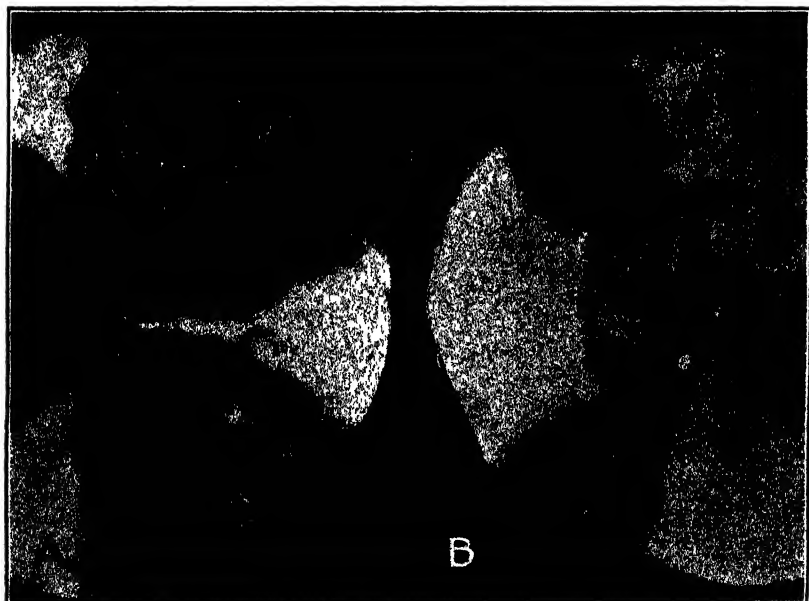


FIGURE 16.—Cross section of Jonathan apple showing type of decay caused by *Aspergillus* forms No. 7 (A) and No. 9 (B)

TEMPERATURE RELATIONS OF ASPERGILLUS FORMS IN CULTURE

Experiments were made to determine the temperature relations of the different forms of *Aspergillus*. Czapeks' solution agar was used as the culture medium. Fresh cultures of the forms were made from single-spore stock cultures, that they might be in a thrifty growing condition before the plantings were made.

On each of two plates three plantings of each of the 11 forms were made and the plates were then placed in the cold-storage room (temperature 0° C.). Two more sets of plate cultures were made in the same manner. One set was placed in the common storage room (temperature 10° to 12°) and the other set was placed in the plant-pathology storeroom (temperature 18° to 22°). These conditions of incubation were the same as those under which the inoculation experiments were carried on. The rate of growth of the 11 forms at temperatures of 10° to 12° and 18° to 22° are given in Table 3.

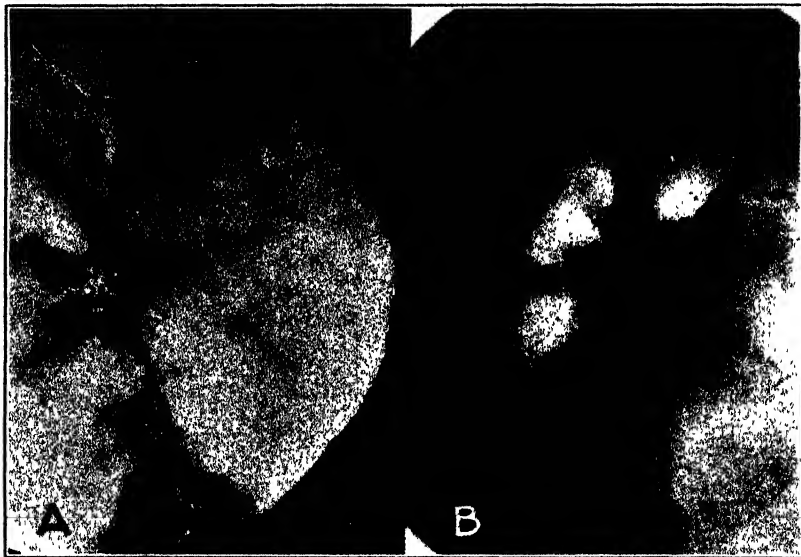


FIGURE 17.—A, Cross section of Jonathan apple showing type of decay caused by *Aspergillus* form No. 10; B, Jonathan apple taken from cold storage showing mycelial growth in calyx end

TABLE 3.—Comparative rate of growth of *Aspergillus* forms on Czapeks' solution agar in common storage and at storeroom temperatures

Aspergillus form No.	Growth in common storage at a temperature of 10° to 12° C.		Growth at storeroom temperatures of 18° to 22° C.		Aspergillus form No.	Growth in common storage at a temperature of 10° to 12° C.		Growth at storeroom temperatures of 18° to 22° C.	
	Average growth per day	Total growth, 24 days	Average growth per day	Total growth, 8 days		Average growth per day	Total growth, 24 days	Average growth per day	Total growth, 8 days
1.....	¹ 0.104	2.5	0.425	3.4	7.....	0.083	2.0	0.375	3.0
2.....	.125	3.0	.4	3.2	8.....	.062	1.5	.463	3.7
3.....	.033	.8	.275	2.2	9.....	.141	3.4	.463	3.7
4.....	.15	3.6	.4	3.2	10.....	.033	.8	.413	3.3
5.....	.166	4.0	.563	4.5	11.....	.158	1.4	.213	1.7
6.....	.062	1.5	.188	1.5					

¹ The figures represent the surface diameter of the rotted areas in centimeters.

At the end of four weeks no growth had appeared on the plantings kept at 0° C. The cultures were then taken to the laboratory and held for several weeks. Growth was somewhat delayed in comparison with that of cultures incubated at room temperature immediately after planting.

All the forms made some growth at temperatures of 10° to 12° C., although the growth was much less than that at 18° to 22°. Some forms grew much more slowly at common storage temperatures than other forms. This is particularly true of forms 3, 8, and 10. Forms 2 and 4 developed much more rapidly at the lower temperatures than did the other forms.

SUMMARY

Apples washed in sterile water with sterile, stiff stencil brushes for five minutes, and the wash water plated out in potato-dextrose agar, yielded from less than 1,000 up to more than 271,000 fungous colonies per apple.

Eleven forms of *Aspergillus* which have been isolated from the surface of normal apples are described and placed in form groups.

Seven of the eleven forms caused decay when inoculated into normal apples. None of the forms caused decay under cold-storage conditions (0°C.). (Forms 2, 5, and 7 caused decay under common storageroom conditions at temperatures of 10° to 12°.) Forms 1, 2, 3, 5, 7, 9, and 10 caused decay under storage conditions at temperatures of 18° to 22° C.

The types of decay caused by the different forms of *Aspergillus* vary greatly. Some forms cause firm and more or less moist rots, others dry and somewhat corky or leathery rots, while *Aspergillus niger* (form 5) causes a very soft and watery rot.

The forms of *Aspergillus* in culture developed more rapidly in storage at temperatures of 18° to 22° C. than at temperatures of 10° to 12°. No growth appeared in any of the forms in cold storage (0°).

CHANGES IN THE PHOSPHORUS CONTENT OF GROWING MUNG BEANS¹

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INTRODUCTION

As one phase of an investigation of the rôle of phosphorus in plants, a study was made of the variations in the content of certain forms of phosphorus which occur in the chief aerial organs of the mung bean (*Phaseolus aureus*) at different stages in its development. The study included plants grown in the field and in test plots treated with superphosphate at the rate of 600 pounds per acre. It is believed that the figures here presented will be of interest to workers in the field of plant chemistry, and that the data may be used to check the results secured from a study of this and other forage crops when grown under controlled conditions of nutrient supply. The phosphorus content is of interest also in relation to the time of harvest, since it is thought that there is a phosphorus deficiency in feeds from certain sections and consequently it would be desirable to harvest the crop when the greatest phosphorus content could be secured.

In a previous paper (8)² from this station the phosphorus fractionation of the mung bean was given together with that of other seeds. Inorganic phosphorus was shown to be a relatively unimportant part of the total phosphorus in the bean, but in the present work with the green plant the inorganic changes were found to be most significant. Because of the color obtained in the solutions, it was impossible to use the iron titrimetric method for phytin; therefore, this determination has been omitted, and the chief comparison will be made between total, inorganic, and organic forms of phosphorus. Since the amounts of lipid phosphorus are small, detailed figures are not given but in their stead are shown the average of the percentages found on the various dates.

WORK OF OTHER INVESTIGATORS

Zlataroff (9) followed the changes in various forms of phosphorus in seedlings of the chickpea (*Cicer arietinum*) grown in water for 25 days. During this time he found that the total and inorganic phosphorus increased, while lecithin, protein, and the soluble organic forms decreased. Not all of these changes continue as plants grow, however, as the present work will show.

André (1) gives figures for phosphorus distribution at four stages in the growth of an annual plant, the poppy. From his analyses he finds that the maximum percentage of phosphorus occurs in roots, stems, and leaves at blossoming time and thereafter decreases. In the fruit phosphorus increases until the plant is nearly mature, after which the change is slight.

¹ Received for publication June 24, 1930; issued December, 1930. Published with the permission of the director of the experiment station.

² Reference is made by number (italic) to Literature Cited, p. 824.

Hartwell and Hammett (7) studied the effect of phosphorus manuring on turnips and found that while increasing the applications of phosphorus increased the total phosphorus twofold, it increased the inorganic phosphorus sixfold.

Greaves and Hirst (4) studied the effect of irrigation on the relation between total and inorganic phosphorus in wheat, oats, barley, and corn. In all cases they found that the increase of total phosphorus, when the amount of water was increased, was chiefly organic in form. They also found that the percentage of inorganic phosphorus was low in these plants and did not vary greatly in different sections.

MATERIALS AND METHODS

The mung bean plants used in this work were grown during the summers of 1928 and 1929. The plants used for the biweekly samplings in 1928 were selected from an unfertilized field of about 2 acres, while those used in the fertilizer studies of 1929 were selected from one-sixtieth-acre plots. All plants were sampled at approximately 1.30 p. m., and samples large enough to give from 50 to 200 gm. of dried material were used except in the case of the first few samplings, when a representative sample could be secured from less material. In no case were less than 10 plants collected for a sample, although in some of the later samplings when the plants were very large not all of the entire 10 plants were used for a sample.

As soon as the samples were cut they were brought in from the field, and the leaves, main stalk, and branches were separated and placed in trays in a hot-air drier. The drier was maintained at a temperature sufficient to dry the samples in eight hours and yet not injure the tissue by charring. After eight hours in the drier the samples were placed in an oven maintained at 110° C., and brought to uniform dryness. They were then ground to pass the fine-mesh screen of the Wiley mill.

The total phosphorus determinations were made according to the directions of the Association of Official Agricultural Chemists (2, *p. 2, par. 6; p. 3, par. 10.1*).

Inorganic phosphorus was determined according to the directions of Collison (3), and while this method may have errors (4) the results are comparative and probably closely approach the real values.

For the estimation of lipid phosphorus the microcolorimetric method of Guerrant (5) was used. The following modifications, however, were made to secure better results (6). Two-gram samples of dried material were extracted with 80 c. c. of the alcohol-ether mixture by shaking for 15 minutes and digesting at 30° C. for two hours, filtering, and making up to 100 c. c. Ten cubic centimeters were used for each determination.

The figures for organic phosphorus represent the difference between the total and the inorganic phosphorus.

Ether extract was secured by extracting dried samples with anhydrous ether in a drip extractor until constant in weight. Even with this procedure it was impossible to secure consistent results with the leaves.

Moisture and ash were determined in the conventional manner.

DATA AND DISCUSSION

Table 1 shows the seasonal distribution of phosphorus in the forms for which analyses were made and the ratio of organic to total phosphorus. This ratio is of value in that it shows how the phosphorus is being utilized and gives some indication as to the seat of active phosphorus changes.

One of the most interesting facts shown in the table is that, with the exception of the third and fourth samplings, when growth was most active, the amounts of inorganic phosphorus are relatively constant, especially so when the marked decreases in other forms of phosphorus are noted.

TABLE 1.—*Phosphorus distribution in mung beans grown in the field, 1928.*

[Percentages based on dry matter]

LEAVES

Date	Moisture	Ash	Ether extract	Phosphorus				Ratio of inorganic to total phos- phorus
				Total	Inorganic	Organic	Lipoid	
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
June 15.....	77.74	12.46	1.07	0.327	0.039	0.288	0.011	0.12
June 26.....	81.00	11.24	1.98	.492	.010	.482		.08
July 5.....	78.33	10.77	.86	.474	.066	.388		.14
July 17.....	77.33	10.60	1.08	.308	.057	.251		.18
July 30.....	77.67	10.05	.74	.261	.048	.213	.01	.18
Aug. 13 ^a	74.30	10.45	.70	.202	.043	.159		.21
Aug. 28.....	74.25	10.07	1.78	.204	.038	.166		.19
Sept. 11 ^b	71.67	9.32	2.12	.122	.030	.092		.25
Sept. 24 ^c	69.60	9.16	1.62	.120	.034	.086		.28
Oct. 17.....								

BRANCHES

Date	Moisture	Ash	Ether extract	Total	Inorganic	Organic	Lipoid	Ratio of inorganic to total phos- phorus
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
June 26.....	90.0	13.91	1.79	0.315	0.074	0.261	0.011	0.17
July 5.....	88.0	10.48	1.70	.288	.061	.227		.21
July 17.....	83.7	8.89	1.47	.256	.090	.166		.35
July 30.....	84.3	7.92	1.28	.197	.089	.128		.35
Aug. 13.....	80.5	5.53	1.98	.149	.070	.096	.009	.34
Aug. 28.....	83.4	5.33	1.16	.163	.077	.096		.41
Sept. 11.....	72.3	4.15	1.10	.085	.070	.035		.59
Sept. 24.....	69.1	3.85	.93	.069	.053	.046		.54
Oct. 17.....	70.6	3.76	1.19	.094	.041	.053		.44

STEMS

Date	Moisture	Ash	Ether extract	Total	Inorganic	Organic	Lipoid	Ratio of inorganic to total phos- phorus
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
June 15.....	85.0	14.95	2.58	0.404	0.056	0.348	0.01	0.14
June 26.....	90.0	12.08	2.20	.431	.068	.363		.16
July 5.....	88.3	7.21	1.85	.373	.097	.276		.26
July 17.....	84.3	5.14	1.19	.210	.093	.117		.44
July 30.....	82.3	5.09	.96	.141	.055	.086	.009	.39
Aug. 13.....	80.25	3.80	.94	.128	.055	.073		.47
Aug. 28.....	72.0	3.55	.95	.102	.062	.040		.61
Sept. 11.....	70.14	3.02	.72	.069	.061	.008		.88
Sept. 24.....	70.61	3.27	.61	.073	.053	.020		.73
Oct. 17.....	69.70	3.25	.71	.074	.041	.033		.55

SEEDS AND PODS

Date	Moisture	Ash	Ether extract	Total	Inorganic	Organic	Lipoid	Ratio of inorganic to total phos- phorus
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Aug. 13.....	83.0	5.88	1.66	0.497	0.070	0.427	.013	0.14
Aug. 28.....	64.56	5.01	.81	.391	.050	.341		.13
Sept. 11.....	60.0	4.98	.46	.415	.058	.357		.14
Sept. 24.....	24.46	4.53	.83	.365	.058	.307		.16
Oct. 17.....	29.05	4.65	.67	.379	.046	.333		.12

^a Pods and beans well formed but still green.

^b Leaves breaking down.

^c Many leaves fallen; others yellow.

The ash determinations are included in the tables to show the relation of total phosphorus to the other mineral elements. In Table 1 it will be noted that the ash content of the leaves falls only about 20 per cent of its value from the June 26 sampling until the last sampling, while the decrease in total phosphorus for the same period was 75 per cent. The reason for this is not apparent, although it was noted when digesting the samples for total phosphorus that there was much more silica in the leaves than in the other organs. This fact may in part be accounted for by the smaller ash content of the other organs, but even this would not seem to account for the entire difference. It was expected when this study was started that a much larger percentage of lipoid phosphorus would be found in the leaves than in other organs, but such is not the case. Since percentages of this form are small and only slight changes occur during the life cycle of the plant, individual figures are not given; instead, the average figure for the early stages and the average for the late stages of growth are given except in the case of the seeds and pods, where a single average is shown.

Another notable fact which is in accord with the idea that the leaf is the seat of synthetic processes in plant metabolism is shown by the relatively large fraction of the leaf phosphorus that is present in some organic form. Since the lipoid form does not account for a large percentage of the whole, it is unfortunate that we do not, in these analyses, have a direct measure of the phosphoproteins of the leaf. Certainly it is true that as long as the leaves are green, organic phosphorus forms constitute the major fraction. The discrepancies shown in the August 13 sampling can probably be explained by assuming a sampling error, since the inequalities run through each analysis at that time.

As would be expected, the values for phosphorus in the stems and branches are similar (Table 1), and show a considerably greater proportion of the total phosphorus in the inorganic form than is found in the leaves. This relationship of the inorganic phosphorus seems to be rather directly related to the type of tissue. The greater the growth activity, the less inorganic phosphorus there is present. Certainly there is no evidence of a transfer of phosphorus from the leaves or branches to the stems. This would seem to indicate a progressive movement to the leaves, since the decrease is least there if the last two samplings made when the leaves had begun to disintegrate are omitted. It is particularly notable that in the case of stalks and branches the decrease in phosphorus is in much the same ratio as the changes in ash content. This fact may be taken as further evidence in favor of the view that the localization of phosphorus is the result of the normal intake of mineral elements and is not due to particular metabolic processes.

Only slight changes occur in the various forms of phosphorus as the plant ages. (Table 1.) This may be due in part, at least, to the great rapidity with which the pods ripen. Since the phosphorus content of the plant varies so little at this stage, a difference of two or three weeks in time of harvesting the beans should not appreciably affect their value as feed.

Table 2 shows a comparison between the phosphorus distribution of plants grown in an unfertilized field (check) and those grown on plots fertilized at the rate of 600 pounds of superphosphate per acre.

In addition to the results reported in this table analyses were made of plants variously fertilized with nitrogen and potassium fertilizers. The results of these analyses are not given since they would not significantly alter the percentages of phosphorus here shown.

Perhaps the most important fact brought out in Table 2 is that the use of this high phosphorus fertilizer did not increase the amount of total phosphorus in the plants so fertilized appreciably above that in the plants grown in the checks, except in the case of the leaves and of the pods and seeds in the late sampling. The season was very dry, however, and this may in part account for the fact that the differences were so small. There was little difference in the appearance of the plots or in the amount of growth. It was supposed that the addition of the fertilizer would increase the inorganic phosphorus in the plant, but, as the figures show, the increase is only in the late sampling and the amount of it is small.

TABLE 2.—*Phosphorus distribution in mung beans grown on checks and plots fertilized with 600 pounds superphosphate*

[Percentages based on dry matter]

EARLY SAMPLING, PLANTS 8 TO 14 INCHES HIGH

Plot	Part of plant	Moisture	Ash	Ether extract	Phosphorus			Ratio of inorganic to total phosphorus
					Total	Inorganic	Organic	
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Superphosphate.	Leaves	80.10	12.86	2.29	0.390	0.051	0.339	0.13
	Branches	86.54	14.36	1.38	.275	.081	.194	.29
	Stems	85.25	13.75	1.05	.228	.062	.164	.27
Check	Leaves	77.77	13.03	2.17	.380	.051	.329	.13
	Branches	85.96	14.07	1.74	.203	.094	.199	.32
	Stems	86.04	11.37	1.24	.237	.076	.161	.32

LATE SAMPLING; SEED PODS NEARLY MATURE

Superphosphate.	Leaves	74.66	11.07	2.08	0.247	0.036	0.211	0.15
	Branches	78.66	6.53	.99	.150	.045	.105	.30
	Stems	76.32	4.95	.67	.142	.046	.096	.32
	Pods and seeds	81.60	5.57	.92	.335	.061	.274	.18
Check	Leaves	75.67	11.64	1.79	.216	.031	.185	.14
	Branches	79.34	7.09	.78	.150	.038	.112	.25
	Stems	76.50	5.44	.68	.148	.040	.108	.27
	Pods and seeds	80.33	5.28	1.73	.300			

SUMMARY

A study was made of the phosphorus content of the various aerial organs of the mung bean as the plant grew to maturity. One set of analyses involved the use of plants grown in an unfertilized field and the other that of plants grown on plots fertilized at the rate of 600 pounds of superphosphate per acre. The determinations for certain phosphorus fractions, ether extract, and ash content are shown.

A most interesting finding is the relatively small change that takes place in the amount of inorganic phosphorus present in any one organ. In fact the amount of inorganic phosphorus in any organ is relatively constant except when the plant is small, and apparently

phosphorus as taken into the plant is metabolized to organic forms for storage.

In the green plant lipid phosphorus constitutes only a small fraction of the whole and varies little at different stages in the growth of the plant. Apparently it is not a storage form of phosphorus in green plants.

At about the usual harvest time there is only a slight change in the total phosphorus content, so that differences of two or three weeks would make only negligible changes in the phosphorus feeding value.

On the basis of quantities present there is no evidence that phosphorus is transferred from the leaves and branches to the stems, and it is assumed that the localization of phosphorus follows the normal ash intake and is not the result of metabolic activities.

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STUDIES ON BACTERIAL CANKER OF TOMATO ¹

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INTRODUCTION

Bacterial canker of tomatoes was first described by E. F. Smith (24)² in 1909. He cultured the causal organism, which he named *Aplanobacter michiganense*, proved its pathogenicity, and made some cultural studies of the organism and histological studies of diseased plants (25, 26). He called the disease Grand Rapids disease of tomatoes, from the location where it was first found, but in his textbook (26) he renamed it bacterial canker of tomatoes. Doidge (11) in the same year used the name tomato canker for another disease occurring in South Africa. Gardner and Kendrick (14) have identified this South African disease with the bacterial spot of tomato and pepper occurring in the United States, caused by *Bacterium vesicatorium*; and the name "bacterial spot" used by them is commonly accepted for this disease in the United States. Kotte (16) suggests that bacterial wilt would be a better name than bacterial canker, since the cracks on the stems are not true cankers; but the existence of another bacterial wilt, caused by *Bact. solanacearum*, and the identification of a cankerlike fruit spot with the disease caused by *A. michiganense* make this name undesirable. It seems justifiable, therefore, to retain the name bacterial canker for the disease caused by *A. michiganense*.

A circular and several notes on bacterial canker have been published by the writer (4, 5, 6, 7, 8). The present paper gives in more detail the results of recent studies.

GEOGRAPHICAL DISTRIBUTION

Bacterial canker is a serious menace to both field and hothouse tomatoes. First described from Michigan in 1909, the disease was later reported from New York, Massachusetts, Pennsylvania, Connecticut, New Jersey, Ohio, and Illinois; but until 1927 it was not known outside of this northern group of States. A severe outbreak occurred in large commercial fields in New York in 1926 (15). In 1927 the disease was reported from Georgia, Utah,³ and Montana; in 1928, from Washington (21, p. 23), California, and Wisconsin; and in 1929 it appeared in Indiana, Mississippi, and Maryland, and more

¹ Received for publication June 30, 1930, issued December, 1930.

² Reference is made by number (italic) to Literature Cited, p. 850.

³ LINFORD, M. B. PLANT DISEASES IN UTAH IN 1927. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 50: 100-101. 1928. [Mimeographed.]

widely than before in California (20). Since the fruit spot caused by *Aplanobacter michiganense* was observed as early as 1917 but was not identified with this disease and was later confused with the bacterial spot caused by *Bacterium vesicatorium*, the distribution is probably wider than here recorded. Certainly Iowa must be included on the evidence of a photograph by Pritchard (unpublished) of typical spotted fruit from Muscatine, Iowa, in 1917.

Bacterial canker has been reported from several foreign countries. Peglion (22) in 1915 reported a disease occurring in Italy which he considered identical with bacterial canker. McLarty (18, 19) noted its occurrence in British Columbia in 1925 and 1926. In 1928 Bisby and Connors (2) found it in Manitoba. Its first appearance in Germany has just been reported by Kotte (16). A disease said to resemble this disease more than the one caused by *Bacterium solanacearum* was reported as occurring in an Australian greenhouse (23) in 1925, but no cultures were made. The statement that the plants recovered with the onset of warmer weather makes its identity doubtful.

ECONOMIC IMPORTANCE

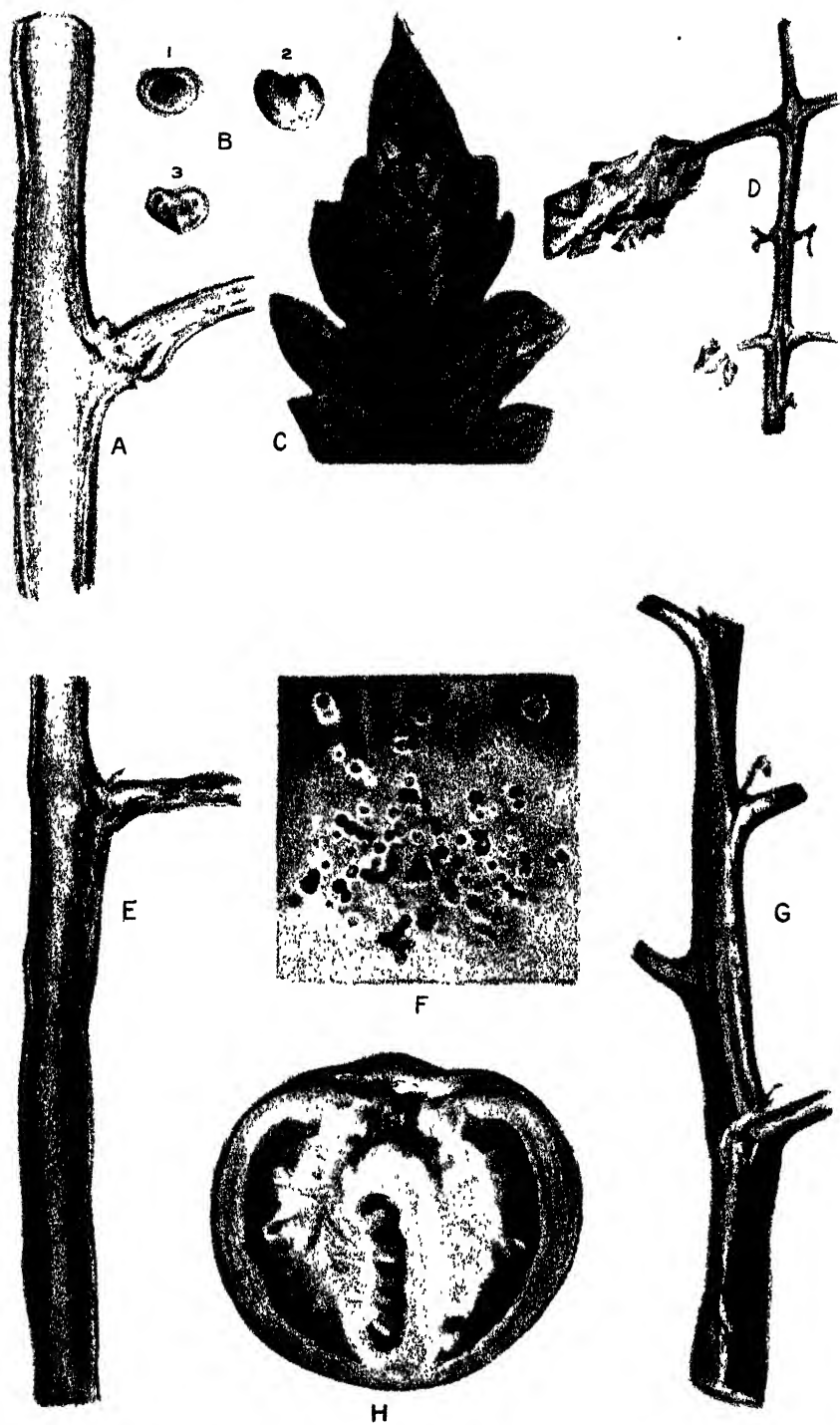
The widespread occurrence of bacterial canker and the fact that it is seed borne and is able to overwinter in the soil under some conditions, together with its very destructive nature, make this one of the most serious tomato diseases known. Field losses range from a trace to 100 per cent in individual fields. In many cases at the time when the crop should be in bearing there are very few living plants left in a field. (Fig. 1.) Such destructive outbreaks have occurred in Michigan, New York, Georgia, and Utah in large commercial plantings, and in smaller acreages in many other places. Fruit spot has caused losses of 25 to 75 per cent of the otherwise marketable crop in some fields in Georgia and Mississippi.

HOSTS

In the field the disease has been found only on tomato, although Smith (25) reported its spread to a spiny Porto Rican weed, *Solanum mammosum*, in the greenhouse. Repeated inoculations have failed to produce any infection on potatoes, eggplants, or peppers of either sweet or Cayenne varieties, or on the common solanaceous weeds *Physalis* sp. and *Datura stramonium*. On the other hand, no variety of tomato tested has been found resistant. The varieties tested include Marglobe, Marvelosa, Norton, Earliana, John Baer, Greater Baltimore, Bonny Best, Chalks Jewel, Stone, Globe, Red Pear, Walter Richards, Gulf State Market, Crystal Springs Market,

EXPLANATORY LEGEND FOR PLATE 1

- A.—Longitudinal section of an infected stem. Early stage showing the yellow or cream-colored infected areas. Note the extensive invasion of the base of the petiole.
- B.—Cross sections of bases of petioles. 1, normal; 2, early infected; 3, late stage of infection, with cavities.
- C.—Leaflet showing leaf spots in several stages.
- D.—Portion of leaf showing a streak on the rachis running out on a petiolule. Leaflets on the opposite side were healthy.
- E.—Section of an infected stem at a later stage. Extensive invasion of the pith with cavities and darker color.
- F.—Bird's-eye spots on green fruit.
- G.—Streaks on a stem, running out onto petioles and beginning to crack open.
- H.—Systemically infected fruit. Bacteria have flooded out from the vascular strands, making them appear broader than when freshly cut.



Ignotum, and a variety of the potato-leaf type. Kotte (16) reports Tuckswood, Triumph, and Lucullus as susceptible varieties.

PHASES OF THE DISEASE

Bacterial canker has two serious phases. The primary phase is a systemic infection which causes the gradual wilting and death of the plant and infection of the seed through the vascular system; and the secondary phase is a fruit spot which, although superficial, renders unsightly a large percentage of otherwise marketable fruit.

SYSTEMIC INFECTION

EFFECT ON THE PLANT

The plant may go down at any stage, from seedling to the mature plant with a set of fruit. The disease usually progresses rapidly, but



FIGURE 1.—Tomato field in Georgia, vacant places caused by bacterial canker

several cases of recovery from early symptoms have been observed. Plants in the field may die before branching or setting fruit. Plants that have attained branching size show decided stunting of one or more branches, often with a thickening of the shortened internodes. (Fig. 2.) Light-colored streaks, sometimes becoming dark, appear on the stems, extending along one or more internodes or up the lower side of petioles or peduncles. (Pl. 1, G; figs. 3 and 4.) Narrower streaks also appear on the upper side of the rachis (pl. 1, D), and on the midribs and veins of the leaflets. These streaks, which follow the line of heavy infection in the cortex, crack open at intervals, forming cankers. (Figs. 3, 5, and 6.) The cankers on midribs and veins, though inconspicuous, are very common and from field evidence appear to cause more secondary infections than do the larger cankers on the stems.

One of the earliest symptoms, more noticeable on staked than on unstaked plants, is a turning downward of the lower leaves, accompanied by curling of the leaflets. An irregular wilting and

shriveling of the leaflets follows. When, as is very common, the infection runs on one side only of the petiole and midrib, a distortion of the leaf results, the leaflets on one side remaining healthy, while those on the other side die. (Fig. 3.) The petioles do not wilt while the leaflets are wilting and shriveling, but they may turn downward. The topmost leaves remain turgid until infection reaches



FIGURE 2.—Tomato plant diseased with bacterial canker, showing stunting and thickening of stem and irregular wilting

them, although in hot, dry weather wilting of the top has been observed; and at times, in the case of very severe infection, a general wilting of the plant occurs when only the lowest leaves show other symptoms.

In Mississippi, following extremely rapid, succulent growth due to abundant rain after drought, the first symptom on the leaflets was the appearance of sharply outlined pale-green spots of collapsed tissue lying between veins, often following the midrib. These areas, which sometimes were more than 1 centimeter wide by several centimeters long, later became gray as the leaflets wilted.

During the very wet weather occurring in Georgia in May, 1928, unusual symptoms were observed in many of the very succulent plants that were succumbing to the disease. Externally the younger parts of infected stems had a clear, water-soaked appearance; and



FIGURE 3.—Leaf showing infection on one side only. Streaks and cankers on the rachis and the midrib of the terminal leaflet

these, as well as adjacent infected petioles, were water-soaked inside instead of being mealy as infected tissues usually are. They showed the typical yellow color. This was the only time that such a condition was observed.

When a diseased stem is cut lengthwise the path of the disease is found to be very clearly marked by a line of creamy white, yellow,

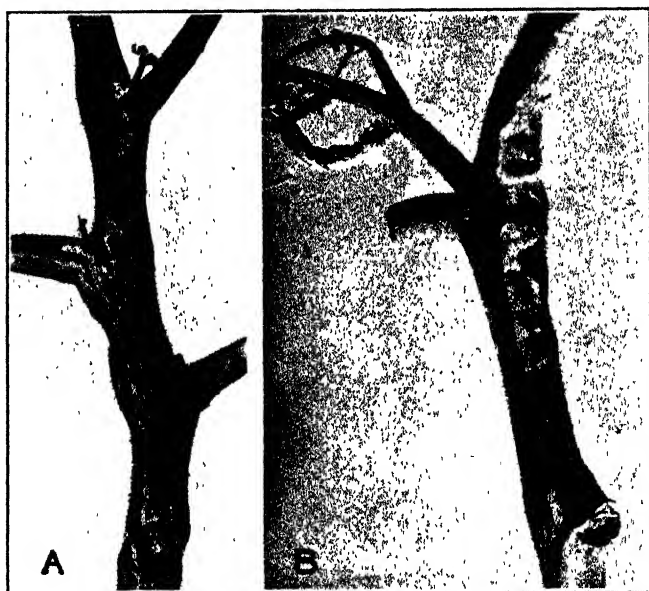


FIGURE 4.—Streaks on stems and petioles. Note the bulging of the stem in A and irregular surface in B over badly disintegrated tissues



FIGURE 5.—Bacterial cankers on tomatoes: A, Canker on rachis, running out on a petiolule; B and C, cankers on stems

or reddish brown, just inside the woody cylinder (pl. 1, A and E), the reddish-brown color appearing in advanced stages. Along this line the pith cylinder is easily separated from the xylem. The discoloration is most conspicuous in the upper part of the plant where the bacteria have spread from the inner vessels of the wood to the more succulent phloem, pith, and cortex. Cavities of greater or less extent are formed in these areas. (Fig. 7.) The pith is often completely disorganized. The base of the petiole is especially susceptible and a cross section taken at this point is the easiest method of diagnosing the disease. Creamy white to yellow, mealy-looking areas in the pith or cortex, sometimes with cavities and in late stages with the red-brown tinge (pl. 1, A, B, and E), are clear evidence of bacterial canker.

Sometimes the root, especially the pith, is completely invaded; more often it shows very slight symptoms easily overlooked by the naked eye, but visible under a hand lens even when infection has taken place from infected soil through the roots; or it may be entirely free from infection. A slightly discolored line in the pith adjacent to the xylem is often the only sign of infection.

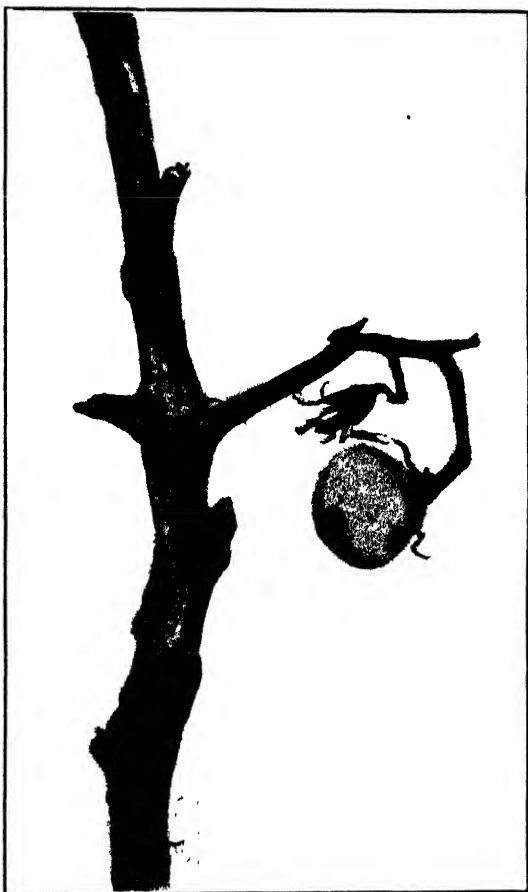


FIGURE 6.—Old cankered stem and a stunted fruit on a cankered peduncle

EFFECT ON THE FRUIT

Infection passes from the stem into the fruit through the vascular system and may be traced by the yellow infected veins directly to the seeds. (Pl. 1, H.) The infection spreads also into the tissues of the placenta and the fleshy covering of the seeds. In the hothouse overripe, heavily infected fruit has occasionally been observed of which the whole interior was disorganized and liquefied while the skin remained intact. Poured plates made from material removed aseptically from the interior of such a fruit gave pure cultures of *Aplanobacter michiganense*. Fruits infected when very young are

stunted and distorted. (Fig. 8, A.) Fruit that develops to nearly or quite normal size shows no external sign of internal infection. Yellow color is observable, however, in the scar made by removing the calyx. Heavy infection of the pedicel just beneath the calyx causes browning and shriveling of the sepals and loosens the attachment of the fruit. The tissues at the junction of pedicel and peduncle are very susceptible and frequently become so disintegrated that the fruit falls when the vine is shaken. Open cankers are common at this joint. (Fig. 8, B.)

In fruits of normal size the seeds do not show external evidence of internal infection, even when there is very heavy infection in the placenta and when the bacteria may be traced by the yellow veins directly to the seeds. Only in a stunted, distorted fruit, infected

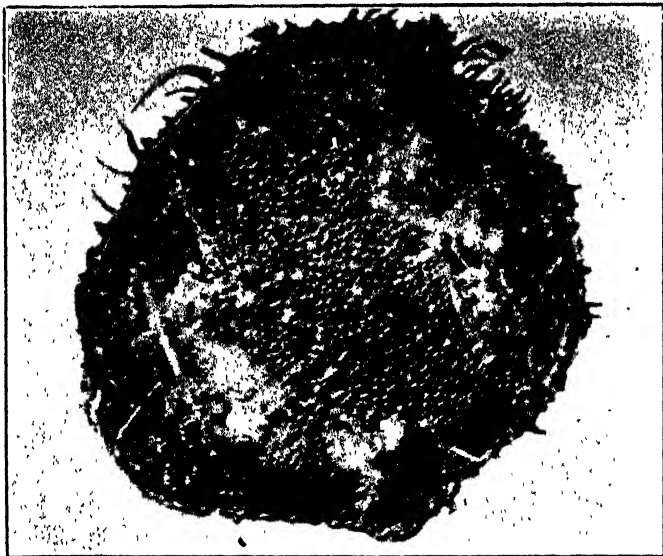


FIGURE 7.—Cross section of a diseased stem showing infected areas (white) and cavities forming in pith, xylem, and cortex. $\times 5$

when very young, are the seeds abnormal. Then they are small, often black, and do not mature. A few such blackened or black-spotted seeds may be found in the larger fruits. Normal-looking seeds and discolored but normal-sized seeds from infected fruit give a high percentage of germination.

FRUIT SPOT

FIELD OBSERVATIONS

Fruit spot of the tomato was first observed by the writer in Georgia in 1928. Previous field studies had been made during the very dry season of 1927. In 1927 systemic infection was very severe in Georgia, but no fruit spot was noted. In the same location the following year systemic infection was again very serious. This was an unusually rainy season, and the idea that drought was a factor in the severity of the disease had to be abandoned, since the systemic infection was as severe as in the preceding dry year. On the first set

of fruit small, round white spots, some with brown centers, were noticed. Specimens were brought to Washington and isolations made. *Aplanobacter michiganense* was obtained in every case. Later the fruit spotting was so widespread in these fields that a large percentage of otherwise salable fruit had to be discarded. The next year (1929) there was a much smaller percentage of both the systemic disease and the fruit spot in these fields.

In 1929 bacterial canker was observed for the first time in Mississippi. (Fig. 9, A.) Fruit spot was more conspicuous than the systemic infection in these fields; that is, there were very few vacant places, but 75 per cent of the fruit that had set was spotted. There had been unusually heavy wind and rain storms, and the large number of

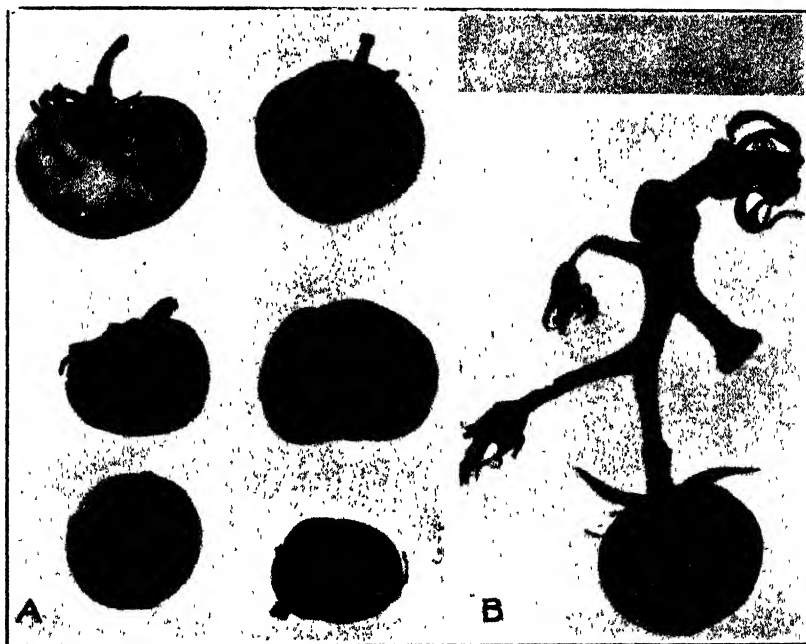


FIGURE 8 — Systemically infected fruits. A, Stunted, distorted fruit, B, infected fruit spur. Note cankers at junction of pedicels and peduncle, which cause the fruit to fall readily

fruits that were spotted on the side toward the prevailing wind gave good circumstantial evidence of spread by wind-blown rain. Open cankers on leaves or petioles could usually be found on the plants in the next row. This was especially significant when there were no cankers on the plant bearing the spotted fruit.

Good evidence that infection was carried from the leaf cankers to the fruit in the drip from rain or dew was obtained in Maryland and New Jersey, where only a small amount of disease was found and where the more conspicuous fruit spots led to the finding of the small leaf cankers just above. Fruit spot has also been traced to infection coming from open cankers on sepals or at the junction of fruit and calyx. Here where moisture is held by the calyx, providing ideal conditions for infection, elongated or irregular brown spots with white margins form along the edge of the scar. In some cases white

areas several millimeters in extent were observed in which a central breakdown had not begun. Groups of typical bird's-eye spots on the

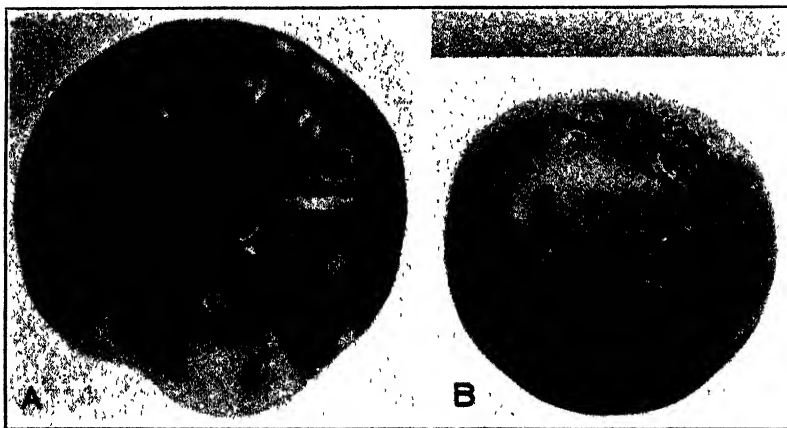


FIGURE 9. - A, Infected sepals, canker with white border running down from point of attachment, and large bird's-eye spots, B, numerous small bird's-eye spots

side of the fruit appear to have resulted from infection borne by drops of moisture from the open cankers under the calyx. (Fig. 9, B.)



FIGURE 10.—Bird's-eye spots on a young green fruit, produced by spraying with *Aplanobacter michiganense*, photographed 16 days after inoculation. $\times 2$

The lesions on the fruit are at first minute, round, snowy white, slightly raised spots occurring singly or in large or small groups. (Fig. 11, A.) As these develop their centers become pustular and

break open with a single rift or with many small cracks, showing a roughened yellow-to-brown surface. (Fig. 10, Fig. 11, B, and Pl. 1, F.) The white persists indefinitely as a conspicuous halo, although in some old spots it disappears partially or completely. Spots may be so numerous as to form large, crusty patches. There is no water-soaking at any stage of development. The spots remain small, not exceeding 3 mm. in width, exclusive of the halo.

ISOLATIONS AND INOCULATIONS

Bacteria were repeatedly isolated from the spots with white halos. Isolations are most easily obtained from fruit spot by swabbing the surface repeatedly with sterile water, removing a group of spots with a sterile knife, and crushing them in a tube of beef broth from which the plates are then poured. Slant agar transfers from the resultant colonies that appeared typical for *Aplanobacter michiganense* were used to make prick inoculations on stems of healthy tomato plants in the hothouse. Typical systemic infection was obtained, the organism exhibiting as much virulence as freshly isolated, authentic isolations of *A. michiganense*. With the same culture fruit spot was produced by lightly swabbing young fruits from one-half to 1 inch in diameter with water suspensions from agar slants, using a small wad of cotton. Later, fruit infection was obtained by spray inoculations with the same isolations. (Figs. 10 and 11.) Reciprocally, fruit spot was readily produced by inoculation with *A. michiganense* isolated from systemically infected stems. White spots one-half millimeter wide were usually noticed from four to six days after inoculation by either swab or spray. The size of the subsequent spot and the rapidity of the development of the roughened center depend on the age of the fruit. The largest spots developed on fruits that were very small (one-half to 1 inch in diameter) when inoculated.

Comparative inoculations were made on young fruits with a culture of *Bacterium vesicatorium* received from Gardner. The resultant spots were always like those described by him. Water-soaking was always present, and no spots with white halos appeared.

HISTOLOGY

Gardner and Kendrick (14) stated that since there are no stomata on tomato fruits and typical infection with *Bacterium vesicatorium* was not obtained by means of insect punctures, bacteria probably penetrate the epidermis through minute rifts in the cuticle or through broken hairs. In this they are doubtless correct. The young fruit is densely covered with two types of very delicate hairs, one a long slender trichome, the other a short hair with a several-celled glan-

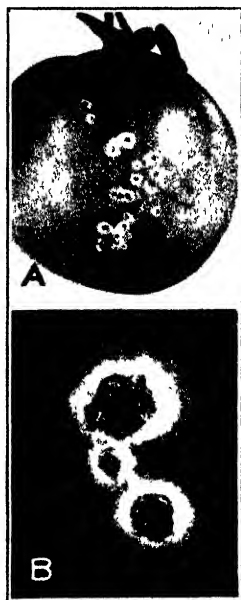


FIGURE 11.—Spots produced by spray inoculation. A, Very young spots five days after inoculation. $\times 5$. B, Enlargement of portion of Figure 10 to show the rough, cankered centers of the spots. $\times 6$

dular head. Sections of fruit with young bacterial canker spots have shown the bacteria in the bases of hairs (fig. 12, A) and in the intercellular spaces of the adjoining subepidermal layers.

Fruit spots remain very superficial, not penetrating as deeply as those caused by *Bacterium vesicatorium*, but otherwise much like

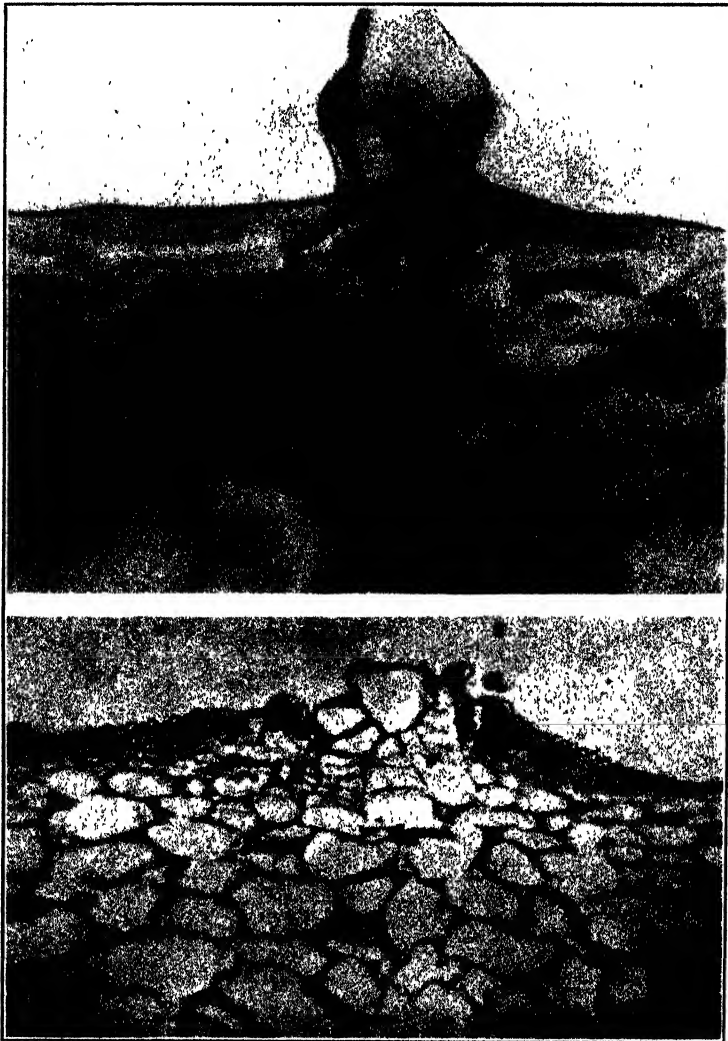


FIGURE 12.—Sections of spots on green fruits. A, Bacteria in the base of an epidermal hair and in the subepidermal tissues. $\times 40$. B, Section through a young spot. $\times 65$

them in development. (Fig. 12, B.) The cells below the infected part multiply noticeably in an attempt to cut it off with a cork layer. There is also a multiplication of subepidermal cells in the region of the white halo, but the white appears to be partly due to the presence of air in the tissues, since it becomes inconspicuous when the specimen is put into alcohol.

EARLIER OBSERVATIONS ON BIRD'S-EYE SPOT¹

In 1918 Coons and Nelson (10) published a brief note on a tomato fruit spot which they called canker, found in several widely separated regions. They described the spots as white, and the single illustration tallies with the fruit spot caused by *Aplanobacter michiganense*. Photographs and notes kindly furnished the writer by Nelson show that this was the predominant type of spot. Coons (9) in a later paper says: "The disease is disfiguring enough to cause serious loss to the crop." His illustration in this case, however, looks like the spot caused by *Bacterium vesicatorium*. Coons and Nelson isolated yellow organisms from both types of spot, but inoculations were made only with the one from dark spots. Nelson's photographs of inoculated fruit give clear evidence that they were dealing with *Bact. vesicatorium*. Their cultures were lost before Gardner and Kendrick studied *Bact. vesicatorium*, so a comparison of the organisms could not be made.

Gardner and Kendrick (12, 13, 14) also found the white spots, which they called "bird's-eye spot." Their illustrations of this type of spot are absolutely typical of bacterial canker. The legend accompanying the illustration states (14): "Bird's-eye spot of tomato. Cause not proved. Possibly a form of bacterial spot." They did not succeed in isolating an organism from these bird's-eye spots, nor did they produce them by inoculation with *Bacterium vesicatorium*. They considered that they were working with the disease described by Coons and Nelson, as they say (13): "The disease as it occurs on tomato fruit has been known for several years. Coons and Nelson, and later Coons, briefly describe this disease as tomato canker." It is interesting that the spot originally called canker proves to be a phase of the disease now bearing that name.

McCubbin (17) in 1918 figures early and late stages of what he calls black scab and which Gardner and Kendrick (13) say appears to be bacterial spot. The figures of the early stage, however, show only white spots, which are typical of bacterial canker spots in the early stage. It would seem, therefore, that McCubbin had both diseases under observation.

SECONDARY INFECTION

Where fruit spot is present in the field it is usually accompanied by spotting of peduncles, pedicels, calyx, and young stems. This takes the form of small, tan-colored, open cankers from 1 to 2 mm. wide. These remain very superficial, and although they occasionally have been found to penetrate deeper and reach the vascular system on inoculated plants, progress is so slow as to be of no practical importance. Leaf spot has not been certainly identified in the field, because of the abundance of fungus spots that confuse the picture. The only place in which leaf spot has been observed to occur naturally and has proved to be caused by *Aplanobacter michiganense* was an abandoned "spotting" bed in Georgia, where the overgrown plants, many with open cankers, were in a tangled mass. (Fig. 13.)

Spotting on stems and leaves has been obtained in the greenhouse by spray inoculations. (Fig. 14.) Infection appears to start fro-

¹The name bird's-eye spot, used by Gardner and Kendrick for this spot, the cause of which they did not determine, should be retained because of its descriptive value.

quently in the large bulbous bases of the giant hairs. Stomatal infection was recorded and figured by Smith (26). Leaf spots are minute and extremely inconspicuous, creamy white, and raised by the mass of bacteria in the tissues. These may remain indefinitely in this condition or dark rings of dead tissue may form around individual spots (fig. 14, A and B, and pl. 1, C) and merge into larger dead areas (fig. 14, C). Occasionally infection reaches the vascular system from such spots, especially from those lying over the very large bundle of vessels on the extreme leaf margin. Where such spread occurs the leaf blade yellows from the margin inward and the veins blacken. (Fig. 14, D.)

SPREAD IN THE FIELD

No positive evidence of field spread of systemic infection by any agency has been obtained. The greenhouse spread recorded by



FIGURE 13.—Naturally infected leaves from an old spotting bed in Georgia. *Aplanobacter michiganense* was isolated from spots on these leaves

Smith has never occurred in the writer's experience. Moreover, the plants throughout the field in many cases succumb at approximately the same time. Slight variations are readily accounted for by the varying times at which infection takes place during the weeks in the seed bed. Spread in the greenhouse by the pruning knife has been reported by Coons (9) and by Williams (28). The disease has not been observed in fields where pruning was practiced, except in Mississippi; and there no convincing cases of spread were observed. Pruning in the field is often done by twisting out the side shoots. This, while not carrying the disease from plant to plant on the knife, leaves wounds for the possible entry of the bacteria. However, the disease progresses downward in the plant very slowly. The bacteria have been traced down from leaf inoculations through the petiole into the stem, but the spread from there is upward. When inoculation is made directly into the stem the spread downward is slow. Kotte (16) has recorded the same observation.

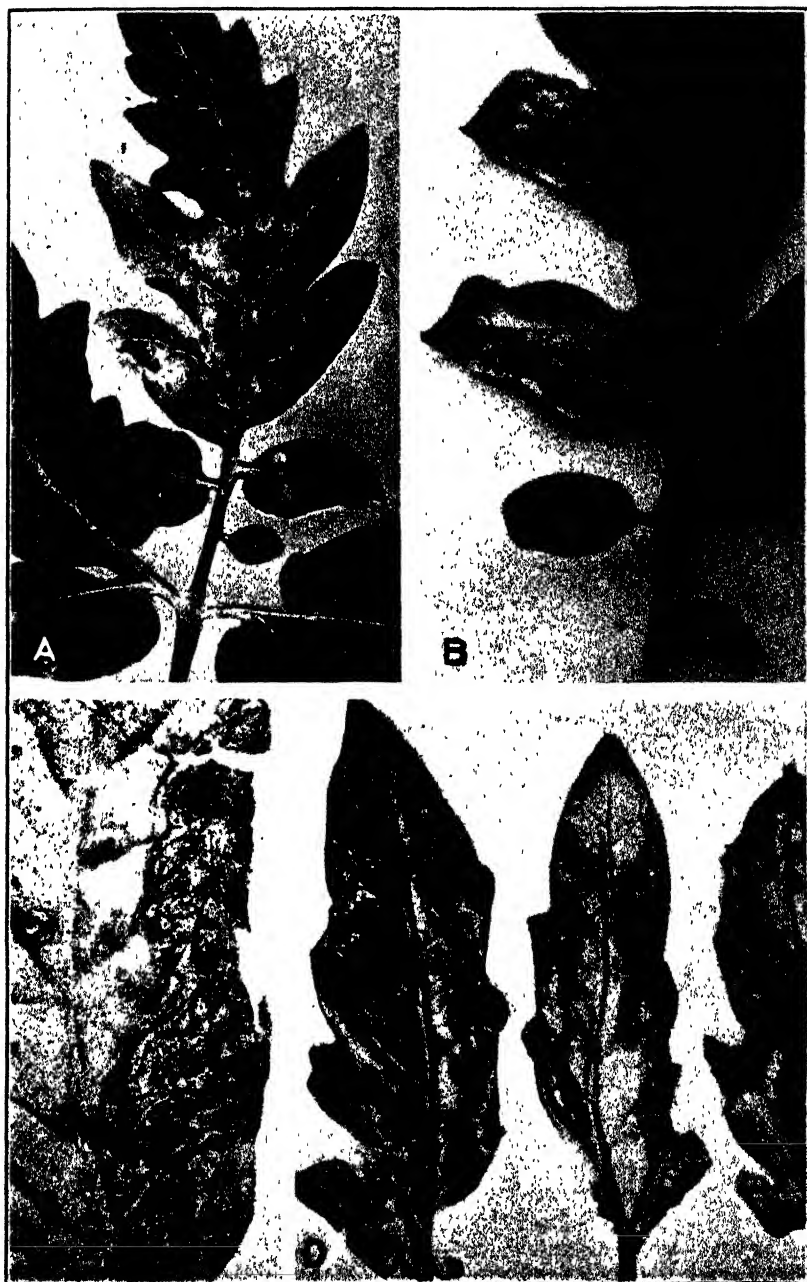


FIGURE 14.—Leaves inoculated by spraying with *Aplanobacter michiganense*. A, Upper surface of leaflet five days after spraying. Natural size. B, Leaflet dying from systemic infection; note blackened veins. $\times 2$. C, Enlargement of a dead area in which small individual spots are distinguishable. $\times 2$. D, Leaflets dying back from marginal infections. Natural size

INTERNAL INFECTION OF SEED

The presence of the bacteria inside the seed coat has been demonstrated by microtome sections and by cultural methods.

Sections of infected seed show that the bacteria lie in the fleshy outer seed coat (fig. 15), which is removed in the seed-cleaning process, and between the layers of the hard seed coat, penetrating the entire circumference of the seed in cases of severe infection. (Figs. 16, 17, and 18.)

Plates were poured from seed taken from fruits that showed infection in the placenta. Since not all seeds are internally infected, 10 or more were used for a culture. The seeds, after the removal of the fleshy, outer seed coat, were soaked in mercuric chloride, 1 to 1,000, for five minutes, rinsed thoroughly in sterile water, and crushed in beef broth. Plates were then poured and *Aplanobacter michiganense* obtained in pure culture. Isolations, identified by inoculations on tomato, have been made from seed 2½ years old.



FIGURE 15.—Section of seed from which the fleshy covering had not been removed, cut through the hilum at right angles to the flattened surface and parallel to the long axis. Bacteria are abundant in the dark areas of the seed coat beneath the hilum and running up both sides of the seed. The fleshy, outer seed coat is heavily infected. \times about 40

MEANS OF DISSEMINATION

Infected seed, and plants grown from infected seed or in infected soil and shipped to other localities, are the means of spreading bacterial canker from place to place. There is also evidence of its having been carried in manure. Seeds are infected both externally and internally. The heavy infection in the placenta of diseased fruits insures wholesale contact of bacteria with the seeds in the process of seed saving. The bacteria are able to survive in a virulent condition eight months or more on the seed or on cover glasses and two and one-half years in dry soil.

EVIDENCE OF DISEASE FROM INFECTED SEED

That disease results from planting infected seed is attested by wide field observations and by experimental planting of seed from infected fruit. From 1 to 5 per cent of infection has resulted in the greenhouse from naturally infected seed and from 21 to 40 per cent from seed dipped in bacterial suspensions, dried, and planted. Field tests in Georgia with seed saved from infected fruit gave 53.4 per cent of cankered plants in 1928 and 30 per cent in 1929;⁵ checks grown from seed known to be clean gave only healthy plants.

⁵ Communication by letter from O. C. Boyd.

A wide discrepancy has been observed in the percentage of diseased plants obtained from seed known to be infected but grown at different times and in different places. For example, seed known to be infected, which gave only 1 per cent of infected plants in the greenhouse in Washington, gave 54.4 per cent in Georgia. This indicates that seed-bed conditions are an important factor in the production of devastating outbreaks of bacterial canker. Since tomatoes are not grown to maturity commercially in the tomato-plant growing region of Georgia and reliable plant growers rotate their fields or use newly cleared pine-lands, soil infection does not appear to be a factor in producing diseased plants in this region. To determine this point clean seed was planted in 1930 in two fields in Georgia from which infected plants were shipped in 1929. No bacterial canker developed on the resulting plants, part of which were grown to maturity in the seed field and part of which (400 plants) were set out in Maryland.

SPREAD IN THE SEED BED

A few primary infections under crowded seed-bed conditions are potentially able to increase the number of diseased plants to 100 per cent, if conditions are favorable. Such spread was found in an old, abandoned plant bed in Georgia during a very rainy season. The plants, which were beginning to set fruit, were in a tangled mass, so that spread was facilitated. Spots were abundant on fruit, stem, and leaf. In many of the plants examined no internal lesions were found in the root or lower stem, although in the tender upper stem they were conspicuous, indicating that systemic infection had resulted through primary lesions on upper stems or leaves. Plants are set in the field or shipped, however, while quite young, and spotting has not been found at this stage. Moreover, severe outbreaks in the field have occurred from hothouse-grown plants where weather conditions do not enter as a factor. In several cases all diseased plants could be traced to one hothouse or coldframe. It would seem that

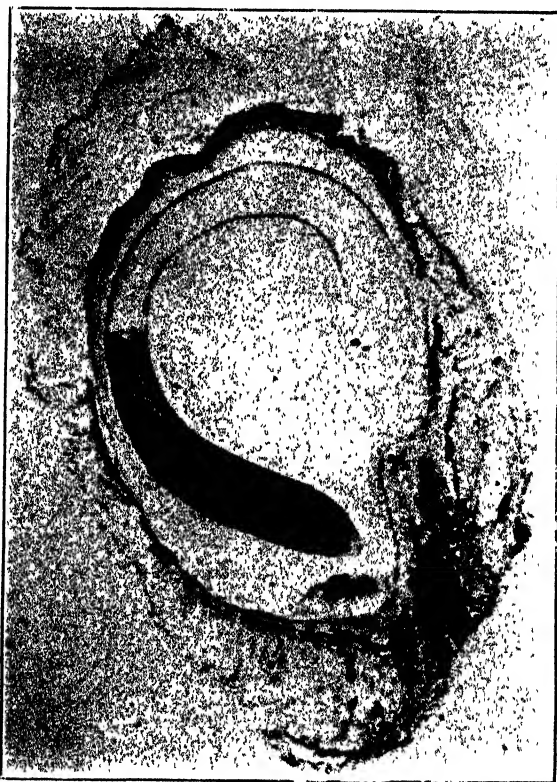


FIGURE 16.—Section of a seed parallel to the flattened sides, showing bacteria (dark areas) in the tissues beneath the hilum and completely encircling the seed. \times about 25

there must be some other cause of spread, possibly insects, in the seed beds. A study of this problem is under way.

SEED TREATMENT

For externally contaminated seed, mercuric-chloride treatment has been found most effective. Such treatment, however, does not control internal seed infection, which is the more serious phase of the disease. For the internal type of infection hot-water treatments were undertaken. A study was first made of the thermal death point of the causal organism at various ages.

THERMAL DEATH POINT OF THE CAUSAL ORGANISM

The thermal death point of *Aplanobacter michiganense* has been found to vary in different strains from 50° to 53° C. This, however,



FIGURE 17.—Enlargement of a portion of Figure 16 to show the position of the bacteria: a, Caulicle; b, endosperm; c, inner wall of seed coat, d, layer of bacteria; e, outer roughened wall of the seed coat. $\times 170$

refers to young 24 to 48 hour old cultures in thin suspensions in beef broth. To ascertain whether it would hold for older cultures or for bacteria massed on the seed and dried there, the following tests were made:

1. Seeds were sterilized in the oven to eliminate saprophytes and thus facilitate the reisolation of *Aplanobacter michiganense*, which is a slow grower. These seeds were then immersed in a suspension from 10-day agar slants of *A. michiganense*, drained, and dried. They were then dropped into sterile water in test tubes and held in a water bath at 55° and 56° C. for 10 minutes and 20 minutes, after first being warmed for 5 minutes at 45°. The bacteria were cultured from these seeds in every case.

2. Bits of the surface agar of slant cultures 20 days old were dropped into beef broth and exposed for various time-temperature periods. Typical bacterial

growth in transfers made from these cultures after exposure demonstrated that the bacteria in this condition are not killed by exposures of 20, 30, or 40 minutes at 54° C. or by an exposure of 10 minutes at 55°.

HOT-WATER TREATMENT OF SEED

Two sets of hot-water treatments of seed were carried on, the first to determine the effect on the seed, the second to ascertain the point at which the bacteria are killed.

1. Freshly cleaned, healthy seeds were tested to determine the point at which injury occurs. The seeds, covered with water in test

tubes, were first exposed in a water bath at 45° C. for 5 minutes, then plunged into water at 50°, 52°, and 54° and kept at these temperatures for 10, 20, 30, 40, 50, and 60 minutes. Germinations were counted in 100 seeds of each lot placed between damp blotters. One hundred seeds of each lot and 100 check seeds were planted in the greenhouse and watched for sturdiness. Although there was slight retardation after the longer time treatments at 54° (3 days at 54° for 40 to 60 minutes), there was no reduction in percentage of germination or vigor of plants in any case. The 50° treatments stimulated earlier germination than took place in the checks. Plants were potted and held until they had reached a height of 8 inches. The same results were obtained with dry seed treated at these temperatures. Similar experiments at 55° and 56°, for 30 minutes and over, produced decided injury to wet, freshly cleaned seeds, both in germination and vigor of plants. Dry seeds were not noticeably injured when treated for 30 minutes at these temperatures but when exposed for longer periods gave a decidedly lower percentage of germination and weaker plants.

2. Seeds from infected fruit were treated with hot water for the various time-temperature periods used in experiments with clean seeds. After such treatment mercuric chloride, 1 to 1,000, was used for 2 minutes to eliminate any saprophytes that might be present on the surface. The seeds were then rinsed in sterile water and crushed fine in beef broth. Since not all seeds are internally infected, 10 or more seeds were used for isolation purposes. The bouillon with the crushed seeds was poured into test tubes containing potato cylinders. Untreated diseased seeds were cultured in the same way, and the resultant yellow bacterial growth tested for its identity. In this way *Aplanobacter michiganense* was cultured from the checks and from seeds treated at 53° and 54° C. for 30 minutes, but not from those treated for 40 minutes. Since 55° is injurious and since commercially it is difficult to hold the water bath at a given temperature without variations of more than a degree, this does not



FIGURE 18.—Section of a seed cut in the same plane as Figure 16 and at a point corresponding to X in Figure 16, showing bacteria massed in the vascular region and more scattered in the adjacent loose tissue. $\times 210$

allow a sufficient margin of safety for general use. Further investigation may show that the method, although impracticable for commercial seed treatment, might be used in cleaning up a seed stock. The only real remedy appears to be in growing clean seed. The only way to obtain clean seed is to select from fields in which there is no bacterial canker.

PERSISTENCE OF BACTERIA IN THE SOIL

Laboratory tests to determine conditions under which the organism can live in the soil have shown tolerance of a wide range of temperature, moisture, and pH values.

Tubes of soil were sterilized, then inoculated with water suspensions of *Aplanobacter michiganense* sufficient to moisten. Half were sealed with paraffin to retain the moisture, the other half were left unsealed and soon dried. Part were held out of doors protected from rain in Washington and part at Geneva, N. Y., from June 15 to April 1. At the end of this period the organism was recovered by cultural methods in virulent form from both wet and dry soil from both places. In another experiment the organism survived two and one-half years in dry sterilized soil exposed to outdoor temperatures in Washington, D. C.

Preliminary experiments have been made in unsterilized soil sealed to retain the moisture and kept at low temperatures in refrigerators. These experiments have demonstrated that the organism will survive five months at 0° F. in soils from different sources with a pH of 5.67, 6.67, and 7.9, respectively. The viability of the organism was determined by inoculation, as it had been found impracticable to isolate *Aplanobacter michiganense* from unsterilized soil. For this purpose the soil culture to be tested was shaken with water for five minutes, allowed to settle, and the suspension thus obtained was used for prick inoculations on young tomato plants. Inoculations were also made by dipping the roots of very young plants in the suspension.

Tomato plants readily become infected when planted in freshly inoculated soil or when inoculum is poured into the soil around them in pots in the greenhouse. However, when plants so infected were allowed to die in the pots, the old vines removed, and young plants set in three months later, no disease resulted. This agrees with the report of Williams (28), who recorded inability to hold the organism in greenhouse soil for even a few months. An attempt to determine persistence in the soil in the open at Washington, D. C., was made. Twenty-four 8-inch pots filled with sterilized soil were buried to their tops in the ground in October. Leaves and stems of infected plants were buried in the upper layers of soil in 14 of the pots. In May the soil in the pots was worked. A water suspension of *Aplanobacter michiganense* was poured into the soil of 6 pots that had not been inoculated in the fall, and the remaining 4 were held as checks. Young Stone tomato plants were set in all of the 24 pots. The plants were watched all summer, but no disease developed in the checks or in the pots inoculated in the fall with diseased plants, although all of those in the freshly inoculated soil succumbed early to the disease. Since this one negative result is not conclusive, the experiment is being repeated, this time without pots.

Negative evidence was obtained in western New York, where for two successive years the disease did not appear in a field that had been a total loss from bacterial canker the preceding year. Further observations and experiments are needed before there can be any certainty in regard to this region.

There is positive evidence, however, that the disease can and does overwinter in the soil in some localities. In Georgia the study of soil hold-over was complicated by the bad field practice of the growers who used infected vines in the compost that was placed under the soil of spotting beds. By this practice almost 100 per cent of the plants became infected. When in 1929 the practice was abandoned there was only from 5 to 10 per cent field infection. This, while indicating hold-over in the soil of fields, is not so large a percentage as might have been expected in fields that had developed 50 to 75 per cent of infected plants the two preceding years. That the bacteria survived composting and were virulent in this compost the second year was demonstrated by Boyd.

Indication of a small percentage of disease from hold-over in the soil was observed in 1929 in California and Utah but was not conclusive because the seeds used were not known to be clean. In Illinois only one diseased plant was found in a half-acre field which had had 25 per cent infected plants the preceding year. Further observations are needed to determine the length of time the infection will remain in the soil. The small percentage of infection so far observed in fields infected the preceding year and the fact that the disease does not attack other crops or the weeds so far tested would indicate that rotation of crops should eliminate the bacteria from the soil. This theory is further supported by the fact that no bacterial canker could be found in 1930 in two fields in Georgia planted in tomatoes for the first time since 1927 when the infection was severe.

CONTROL

Control of bacterial canker is a difficult problem. Seed infection, soil infection, and spread in the seed bed must all be taken into consideration. Where the disease has not occurred, the use of clean seed is the first essential for safety. Seeds brought into disease-free areas for breeding purposes should be grown in separate seed beds, the trial plots carefully inspected, and any infected plants burned.

Seed should not be saved from fields in which even a small percentage of bacterial canker is found. Seed not positively known to come from clean fields should be treated with mercuric chloride, normal Semesan solution, or Semesan Jr. dust. This will eliminate surface infection and is desirable from the point of view of other tomato diseases, but no treatment is known which will destroy internal infection, under commercial conditions, without danger of injury to the seed.

Where the disease has occurred, rotation of crops also is essential. The number of years that the bacteria will live in the soil under field conditions when rotation is practicable has not yet been demonstrated. The small percentage of diseased plants on fields infected the preceding year, the absence of the disease from two old fields after two years in other crops and the fact that other crops and solanaceous weeds are not attacked make this a promising method. Where ro-

tation is impracticable, the development of a resistant strain of tomato would be desirable. The removal and destruction of diseased vines to reduce the amount of infection in the soil would also be desirable under these conditions. Kotte (16) suggests that infection through roots broken in transplanting may be prevented by dipping the roots in a 0.5 per cent solution of Uspulun thickened with soil to the consistency of a thin mud. A preliminary experiment was made in the greenhouse with Kotte's method, in which normal Semesan solution was used instead of Uspulun when setting young plants in heavily inoculated soil. This method resulted in a 20 per cent infection in the treated plants as compared with an 80 per cent infection in the checks.

Care should be taken in the sanitation of the seed bed. Williams (28) reports satisfactory control of the disease in greenhouses by sanitation but gives no details. No infected refuse, either vines or spotted fruit, should be returned to the soil or allowed to reach the compost heap or the barnyard. The soil of old beds in cold frames or hothouses should be replaced with clean soil from fields where tomatoes have not been grown, or it should be sterilized by steam. Hothouses should be fumigated. In the South, where seeds are sown in the open, fields to be used as seed beds should be burned over before plowing to destroy insects in plant refuse. Fall plowing would also aid in decreasing the insect population. A field should not be used two successive years for plant growing for the best results from several points of view.

Very few plants show any sign of infection at the time of setting in the field. Inspection for disease at this stage would be impracticable.

THE ORGANISM

VARIANTS

An albino strain has been isolated from single-colony cultures of *Aplanobacter michiganense* (8). So far as known, such a variant has not been reported for any other plant pathogen. Brown (3) found rough, rubbery, round white colonies on reisolation plates of *Bacterium beticolum* with which she obtained infections on sugar beets, but she did not record their cultural identity with that organism, nor did they appear in single-colony cultures of the typical yellow organism. White variants that do not revert to the typical color have been described by various workers for bacteria in other groups, notably *Bact. prodigiosus*, *Bact. violaceum*, *Staphylococcus aureus*, and several saprophytes from plants.

White streaks were observed in the surface growth on old agar stab cultures at three different times and with isolations from different places. On one occasion plates were poured from the white part of a culture of a strain isolated four years previously, and both white and yellow colonies appeared. These were alike except in color. Transfers from white colonies were found to be nonmotile and Gram-positive. No further work was done at that time and the cultures were lost. When in 1929 the same phenomenon occurred in a strain held two years on culture media, white colonies were again isolated. These were tested as before and also used for inoculating tomatoes. Prick inoculations were made in the stem and swab inoculations on young green fruits. In both cases typical lesions were produced,

the organism proving as virulent as the typical yellow form. Reisolations were made from petioles at intervals of 12 days, 6 weeks, and 2 months after inoculation, from near the point of inoculation and from the upper limits of the bacterial invasion. In all cases only white colonies appeared on the plates. In cultural tests on potato, litmus milk, Thaxter's potato agar, gelatin, beef broth, nitrate broth, and starch agar, the white form resembled the yellow form except in color. No yellow pigment was produced at any time.

Variants of another type were observed on whey agar and Thaxter's potato agar. On both of these agars containing sugar, on which the typical colony growth is abundant, fluid, and spreading, varying percentages of small, round, very convex colonies have appeared. These remain small and never spread. When replated on these agars the round type gives only round colonies; the spreading type may throw off a few round colonies. Replated on beef agar they are indistinguishable. The round colony has appeared in small numbers in young isolations but is much more common in isolations that have been on culture media for some time. In one 10-year-old strain only the round type was present. In this case no infections could be obtained. Round colonies of younger isolations are very weakly parasitic and weak in their reactions on culture media as compared with the spreading strain. They have not, however, lost the power of fermenting sugars.

LOSS OF VIRULENCE

Smith (25) has reported that *Aplanobacter michiganense* loses its virulence in the course of several years. Tests with various isolations—three of 1924, three of 1925, and one of 1926—were used in 1929 to make prick inoculations in the upper stems of large plants. All except two of the 1924 isolations showed symptoms of infection by the tenth day, and the disease developed rather slowly. Inoculation of the stems of young plants with the same cultures produced rapid and complete destruction of the plants, with typical symptoms. Virulence is therefore not lost by five years on culture media. As noted under "Variants," a 10-year-old culture failed to give any infection.

MORPHOLOGY AND STAINING REACTION

Aplanobacter michiganense is a small, nonmotile rod, measuring 0.6 to 0.7 μ by 0.7 to 1.2 μ , and occurring singly or in pairs. It is definitely Gram-positive. No spores have been observed. It stains readily with the usual bacterial stains. Capsules occur in agar cultures.

CULTURAL CHARACTERS⁶

BEEF AGAR.—Growth in beef agar is slow, moderate, opaque, smooth, glistening, pale yellow becoming mustard yellow,⁷ and not opalescent. Colonies on beef-agar plates may be seen under the hand lens on the second day but usually are not visible to the naked eye until the third day. They are then very minute, 1 mm. or less in diameter, very pale yellow, and very convex. By the fifth day they have reached a diameter of 2 to 3 mm., are round with an entire margin, smooth, shining, of butyrous consistency, opaque, pale yellow, and show no markings by either reflected or transmitted light. In 12 days colonies on thinly sown plates may attain a diameter of 6 mm. At this time they are bright yellow

⁶ Unless otherwise stated, all beef media were made with beef infusion and had a pH value of 7.0 to 7.2.

⁷ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C.

and very viscid. Growth on the surface of agar slabs is round and restricted, attaining a width of 7 or 8 mm. by the eighth day. The stab growth is saccate and granular, visible to one-third the depth of the agar. On slant agar there is moderate growth, not running down into the V. On beef-extract agar growth is less abundant, paler (straw yellow) and thinner than on beef-infusion agar; it is opalescent and does not become viscid.

THAXTER'S POTATO AGAR.—On this medium growth is very abundant, fluid, long continued, and paler than on beef-infusion agar, though not so pale as on beef-extract agar. By the sixteenth day colonies may attain a diameter of 15 mm. and show no sign of drying down but remain thick and fluid looking, revealing a flocculent interior by transmitted light. Bottom colonies are thin, opalescent, and about 2 mm. in diameter. In slants the growth flows down, filling the V to a depth of 1 cm. or more, and leaving only a thin layer of growth on the slant. The mass of growth in the V is as deep in color as growth on beef-infusion agar. Large lenticular, granular bodies, looking like giant buried colonies, appear in the surface layer at the base of the slant in about two weeks.

BEEF BROTH.—Clouding is slow and moderate, beginning in about 48 hours. A pale-yellow rim begins to form in patches by the sixth day and is complete by the seventh or eighth day. Occasionally in undisturbed cultures a delicate incomplete pellicle is formed.

POTATO.—On potato cylinders growth is moderate, mustard yellow, smooth, glistening, and spreading, but not covering the entire surface of the potato. The water remains clear, with a moderate precipitate. The potato is grayed.

SYNTHETIC MEDIA.—Cohn's solution takes on a slightly milky appearance, but shows no rolling clouds on shaking. In Uschinsky's solution clouding is weak after 10 days' growth and only moderate, with a small amount of precipitate and with neither rim nor pellicle, at the end of two weeks. Moderate clouding occurs in Fermi's solution.

PHYSIOLOGY

LIQUEFACTION OF GELATIN.—Gelatin is very slowly liquefied at 18° to 21° C. In one test the surface growth after four days was about 4 mm. wide, lying in a dry depression 3 mm. deep. Two weeks later the gelatin was liquefied to a depth of 8 mm. and by the end of the fourth week the stratiform liquefaction was 12 mm. deep. The fluid was clear, with a heavy layer of yellow precipitate on top of the solid portion. No further liquefaction took place during the following month. In other tests evaporation kept pace with liquefaction so that only a dry pit was formed.

RELATION TO FREE OXYGEN.—The organism is aerobic. It does not grow in the lower end of slabs in beef agar or Thaxter's agar or in the lower half of shake agar cultures. No clouding occurs in the closed end of fermentation tubes.

FERMENTATION OF SUGARS.—Tests were made on beef-extract agar slants with bromocresol purple as indicator and 1 per cent, respectively, of the following carbohydrates: Sucrose, dextrose, galactose, levulose, maltose, lactose, glycerin, and mannit. The slants were streaked from agar cultures. In sucrose and levulose, acid was evident at the upper end of the slant after 24 hours. Two days later the whole of the slanted part of the culture was acid in sucrose, levulose, dextrose, and galactose. In maltose the slant was acid on the tenth day. On the fifteenth day the medium was acid throughout in all of these sugars except maltose, which never became as acid as the others. An alkaline reaction started on the seventeenth day and 10 days later the medium was alkaline throughout. In lactose, glycerin, and mannit there was a very slight color change, indicating an acid reaction, after two weeks' growth. Phenol red was then used as an indicator in the same kind of agar with each of these three carbohydrates. Acid was evident on the fourth day in glycerin and mannit, and on the tenth day the whole of the medium was acid. The lactose cultures began to show acid on the tenth day and were acid throughout on the seventeenth day. In fermentation tubes containing these carbohydrates in peptone water, the closed end does not cloud and no gas is formed.

REACTION IN MILK.—There is no evidence of growth in milk for several days. By the fifth day a yellow rim and surface layer of yellow growth 3 mm. deep has formed. These gradually increase to a depth of 1 cm. The milk remains fluid for about a month, although a soft curd may form during this period. In older cultures a solid curd forms with more or less separation of a clear yellow whey. Reduction of litmus is slow, beginning about the eighth day throughout the medium, and is complete in three weeks. No acid reaction appears before reduction has taken place, but the medium turns red when the cultures are 6 to 8 weeks

old. Reduction of methylene blue proceeds from the bottom upward, beginning on the third day, and is complete in 7 to 10 days. No crystals are formed.

HYDROLYSIS OF STARCH.—On starch-agar plates streaks which had made good growth were flooded with iodine when 7 days old. This produced a line 12 mm. wide around the growth, varying from deep purple in the outer portion to pale purple in the inner part nearer the growth, and in some cases colorless in the 1 mm. adjacent to the growth.

REDUCTION OF NITRATES.—Nitrates are not reduced. Tests were made in nitrate-bouillon cultures with the starch potassium iodide-sulphuric acid test and with the α -naphthylamine-sulphanilic acid test, and in the series of special media recommended in the Manual of Methods (27) for organisms giving a negative reaction in the simpler tests.

OPTIMUM pH FOR GROWTH. In a series of broths made with beef infusion and Bacto-peptone, ranging from pH 4.4 to 9.6, growth was evident on the second day at pH 6.1 to 8.3 and was best at pH 7.7 to 8.3. By the fifth day there was clouding at pH 9.0 (at this time the checks had a pH value of 8.6), but there was no growth in the acid range beyond pH 6.1. By the seventh day a rim had formed in the culture at pH 7.5 to 8.2, but not in the others. Berridge (1) records growth with this organism at pH 5.0 to 9.2 in peptone broth made with Jardox. On agar slants growth was decidedly heavier at pH 7.6 and 7.9 than at pH 6.9. On agar plates 2-day-old colonies were barely visible at pH 7.0, whereas they were 1 mm. wide at pH 7.9.

TOLERATION OF SODIUM CHLORIDE.—The organism is sensitive to sodium chloride. Growth is moderate in beef broth at pH 7.0 containing 1 per cent NaCl, weak in broth containing 2 per cent, and does not occur in broth containing 3 per cent.

OTHER PHYSIOLOGICAL REACTIONS.—*Aplanobacter michiganense* does not form indol or hydrogen sulphide. There is slight ammonia production in beef-peptone media, as shown on sugar agars containing bromocresol purple and by strips of filter paper dipped in Nessler's solution and inserted in beef-broth cultures.

TEMPERATURE RELATIONS.—The thermal death point ranges from 50° to 53° C. with different isolations. Tests were made with beef-infusion broth at pH 7.8, inoculated from 24-hour-old beef-broth cultures that had been heavily enough inoculated from an agar slant to cloud well in that time. Smith (25) reported no growth after exposure at 47°. The best growth is made at 25° to 27°. There is weak growth at 33° and moderate growth at 29°. No growth occurs in beef broth at 35° or 37°, but cultures were not killed by exposure for 10 days at these temperatures, as there was prompt clouding on removal to room temperature. Very slow clouding occurs at 1°. The organism has withstood year-round outdoor temperatures in Washington, D.C., and Geneva, N.Y.,⁸ in sealed test tubes of sterilized soil, both moist and dry.

RESISTANCE TO DESICCATION.—*Aplanobacter michiganense* has survived eight months' drying on seeds and on cover glasses⁹ and 2½ years in dry, sterilized soil without complete loss of virulence.

QUICK DIAGNOSIS

Slow-growing yellow colonies from typical lesions appearing on the third day on beef-agar plates, made up of small nonmotile, Gram-positive rods, may be safely diagnosed as *Aplanobacter michiganense*.

BRIEF CHARACTERIZATION

Aplanobacter michiganense is a short, nonmotile, Gram-positive, nonacid-fast rod occurring singly or in pairs, 0.6 to 0.7 μ wide by 0.7 to 1.2 μ long. It forms round, opaque, mustard-yellow colonies on beef agar; clouds beef broth slowly and forms a pale-yellow rim; has very weak diastatic action; liquefies gelatin very slowly; forms acid without gas from dextrose, sucrose, galactose, levulose, and maltose, and a very slight acid from lactose, glycerin, and mannitol; does not reduce nitrates; causes very slow coagulation of milk without peptonization; reduces litmus and methylene blue in milk but does not form tyrosine crystals; produces ammonia but no indol or hydrogen sulphide; is aerobic; grows best at pH 7.5 to 8.2; withstands drying for 8 months on cover glasses and 2½ years in soil; optimum temperature is between 25° and 27° C.; thermal death point, 53°. Pathogenic to tomatoes.

⁸ This result was obtained through the cooperation of J. G. Horsfall of the Geneva station.

⁹ Tests on seed and cover glasses were not carried beyond 8 months, but the organism has been isolated from the interior of 2½-year-old seed.

SUMMARY

Bacterial canker has become more widespread and destructive in important tomato-growing sections in recent years. It causes the death of the plants at any stage from seedling to mature plant with a set of fruit. Losses range from a trace to 100 per cent. The disease is characterized by stunting of the plants; a gradual wilting and irregular dying of the leaves; streaking and cracking open of the stems, petioles, and veins; and the final collapse of the whole plant. The bacteria enter the fruit and penetrate the seeds through the vascular system.

During wet seasons bacterial canker also causes a very disfiguring fruit spot which renders the fruit unmarketable but does not cause it to rot. The spots are small but numerous and are characterized by noticeable white halos. Aside from this fruit spot there is no serious field spread.

The causal bacteria are disseminated on and in the seed. External disinfection should be practiced. Hot-water treatment has been found impracticable under commercial conditions, since temperatures that kill the bacteria are too close to the point at which injury to the seed occurs. According to present information, absolutely clean seed can be obtained only by selecting seed from fields free from this disease. Seed-bed sanitation is important, since the percentage of infection from infected seed is in part determined by seed-bed conditions that cause spread from plant to plant. These conditions are under investigation. Rotation of crops should be practiced, since it has been demonstrated that the bacteria are able to overwinter in the soil in some parts of the country.

The cultural and physiological characteristics of *Aplanobacter michiganense*, the causal organism, are given. Two variants, one an albino, are described.

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A COMPARATIVE STUDY OF THE STEM EPIDERMIS OF CERTAIN SUGARCANE VARIETIES ¹

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INTRODUCTION

A casual examination of the anatomical structure of the stem epidermis in certain varieties of sugarcane revealed, among even the most uniform specimens, differences that seemed to merit more detailed study. Such differences, if established, would, it was hoped, supply diagnostic characters of value in classifying varieties and in determining relationships. Representative varieties of four species of sugarcane (*Saccharum officinarum*, *S. sinense*, *S. barberi*, and *S. spontaneum*) and of several species hybrids which were examined seemed to offer a sufficiently wide range of material within the genus to justify a preliminary study for the purpose of establishing whether these structures might be useful for the purpose.

The existence of certain varietal differences was already known to Wieler,² who, in studying the structure of the sugarcane stem, examined the epidermis of several varieties and noted that in certain canes the siliceous epidermal cells were very abundant while in others they were much fewer in number. Wieler also described in detail the structure and development of these siliceous cells, but his conceptions were greatly modified in a later investigation by Frohnmeier.³ The observations of Wieler were extended by Mameli de Calvino⁴ in her study of the anatomy of Cuban canes. She noticed that varieties differed in the size of the different types of epidermal cells, the thickness of the walls, and the distribution of the stomata.

MATERIALS AND METHODS

The material for the present investigation was obtained from the fields of the Sugar Plant Experiment Station at Canal Point, Fla. Heinemann,⁵ in her study of the epidermis of corn, found great variation in the composition of the epidermis of the different internodes. In order to eliminate the effect of similar differences in sugarcane, special care was taken to select material that had reached the same stage of development. It was found that mature internodes which had reached their final length and were no longer covered by the

¹ Received for publication June 28, 1930; issued December, 1930.

² WIELER, A. BEITRÄGE ZUR ANATOMIE DES STOCKES VON SACHCHARUM. Beitr. Wiss. Bot. B. 2: 41-164, illus. 1898.

³ FROHNMEIER, M. DIE ENTSTEHUNG UND AUSBILDUNG DER KIESELZELLEN BEI DEN GRAMINEEN. 39 p., illus. Stuttgart. 1914. (Bibliotheca Botanica, Heft 86)

⁴ MAMELI DE CALVINO, E. ESTUDIOS ANATOMICOS Y FISIOLOGICOS SOBRE LA CAÑA DE AZÚCAR EN CUBA. Mem. Soc. Cubana Hist. Nat. "Felipe Poey" 4: 156-211, illus. 1922.

⁵ HEINEMANN, K. ZUR KENNNTNIS DER OBERHAUT AM MAISSTENGEL. Bot. Centbl., Beihefte 42: 111-159, illus. 1925.

protecting leaf sheaths were best suited for a comparative study; and, since the middle portion of the internodes showed the greatest uniformity in the appearance and distribution of the various epidermal structures, material was always taken from this region. On the average 10 individual samples of each variety were tested, and it was noted that the variation in the different specimens of a single variety was but slight and did not exceed variations observed in different fields of a microscopic preparation. It should be understood, however, that the tests were based on one season's crop and that practically all of the material came from the same field. It is therefore possible that under changed environmental conditions a somewhat different epidermal picture might be obtained; but because of the conservative nature of these structures such variation is not likely.

The epidermis was removed by the aid of Schulze's maceration fluid, but instead of using the prepared reagent as recommended by Grob,⁶ the writer adopted the following procedure, which gave most satisfactory results. A piece of epidermis with the adhering cortical and fibrous tissue was cut from the central part of an internode and placed in a test tube containing several crystals of potassium chlorate and a few cubic centimeters of concentrated nitric acid to which a few drops of water had been added. The mixture was carefully brought to a boil, and after a few seconds, as soon as the epidermis separated off in the form of a thin pellicle, the contents of the test tube were emptied into a Petri dish partly filled with water. The epidermis was then mounted on a slide and stained with chloriodide of zinc. If the maceration process is interrupted at the right point, the epidermis stains a bright blue; but if the maceration is not successful, the stain will not take at all or the differentiation will not be satisfactory.

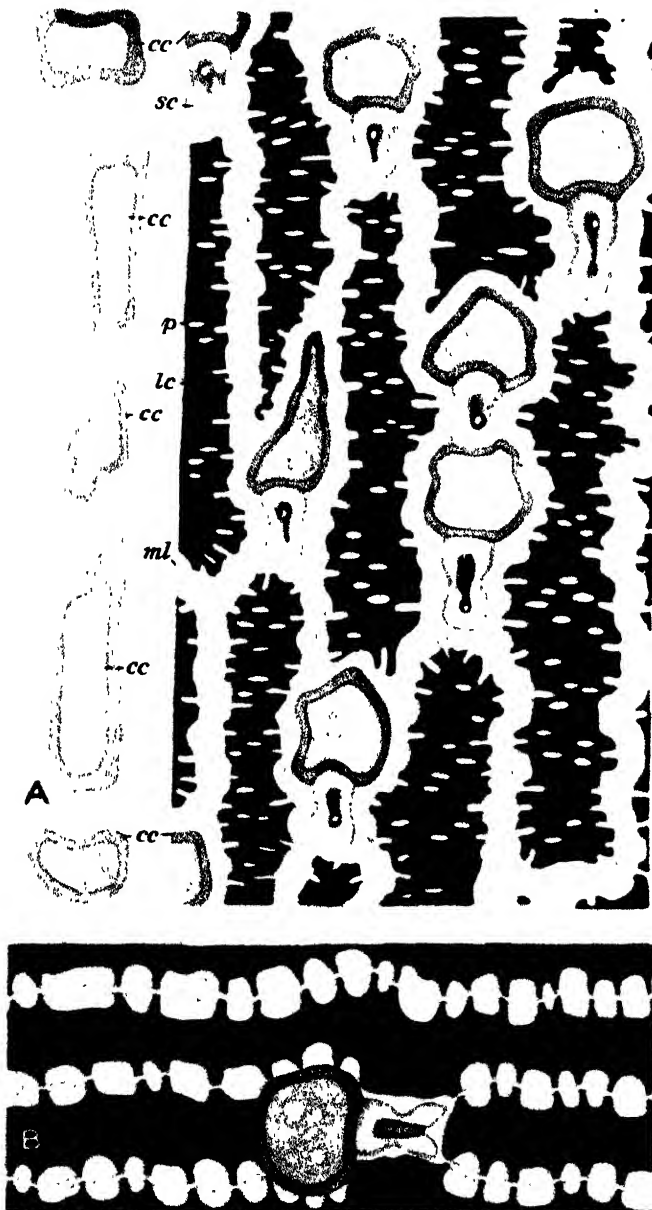
The number of short-cell groups in a square millimeter was determined by projecting the stained preparations on a screen of ample size. This was done in order to estimate their relative number as accurately as possible regardless of irregularities in distribution.

STRUCTURE OF THE EPIDERMIS

When the stem epidermis is removed in the manner described and is properly stained with chloriodide of zinc, it appears under the microscope as made up of various kinds of cells, disposed, however, in a remarkably uniform pattern. Two distinct types of cells alternate with each other—elongated rectangular cells and short cells which occur singly or in pairs. (Pl. 1.)

The long cells, which constitute the greater part of the epidermal cells, form 4-sided prisms. They vary greatly in length, and their end walls, though commonly straight, are often pointed. The variation in width is less evident but becomes quite a factor when different varieties are compared with one another. The walls of the long cells have an undulated, strongly silicified middle lamella, which, in the stained preparation, appears as a pure-white wavy line. The thickening layers are also undulated but not uniformly at all points (fig. 1),

⁶ GROB, A. BEITRÄGE ZUR ANATOMIE DER EPIDERMIS DER GRAMINENBLÄTTER. 122 p., illus. Stuttgart 1896 (Bibliotheca Botanica, Heft 36.)



Litho. A. Hoehn & Co., Inc.

A.—Surface view of epidermis of Louisiana Purple stained with chlorotoluidide of zinc. $\times 1,000$.
cc, Cork cell; ml, middle lamella of long cell; p, pit; lc, long cell; sc, silica cell

B.—Surface section of epidermis of Cayana. $\times 1,000$. Notice the large number of pits in the walls of the long cells

and the degree of silicification is somewhat less. The outer wall of these cells is very thick, strongly silicified and suberized, and possesses a more or less strongly developed cuticle. The inner wall is much thinner than the outer one, stains a bright blue in the preparation, and is pierced by numerous pits. (Fig. 2.) Pits are also found in the lateral walls but are wanting in the outer one. Occasionally the lumen of the cells is filled with calcium oxalate in the form of crystal sand.

The short cells typically occur in pairs, one member of which stains with chloriodide of zinc a golden yellow and is known as a cork cell; the other member of the pair appears a glistening white and is known as a silica cell. Often, instead of two short cells constituting a group, a larger number is found. (Fig. 3.) This increase may be brought about by the omission of a long cell, so that two short-cell groups join each other directly. Sometimes there are one or two extra cork cells present, and thus the group may be greatly enlarged. While an increase in the number of short cells in a group is the more common occurrence, it may happen that by the omission of the silica cell the short-cell group is reduced to one member. This situation is common in some varieties and constitutes, as will be seen later, a valuable diagnostic character.

The cork cells have relatively thin walls, suberized and silicified, and a large lumen which usually contains a solid deposit of some organic substance. Though variously shaped, in certain varieties they may be remarkably uniform, being usually broadly reniform with the greater dimension parallel to the long axis of the stem. Frequently they are square or rectangular, especially in varieties in which the silica cells are few in number or wanting. They may also be trapezoidal, triangular, or much elongated (pl. 1, A) with end walls square or pointed like thick-walled

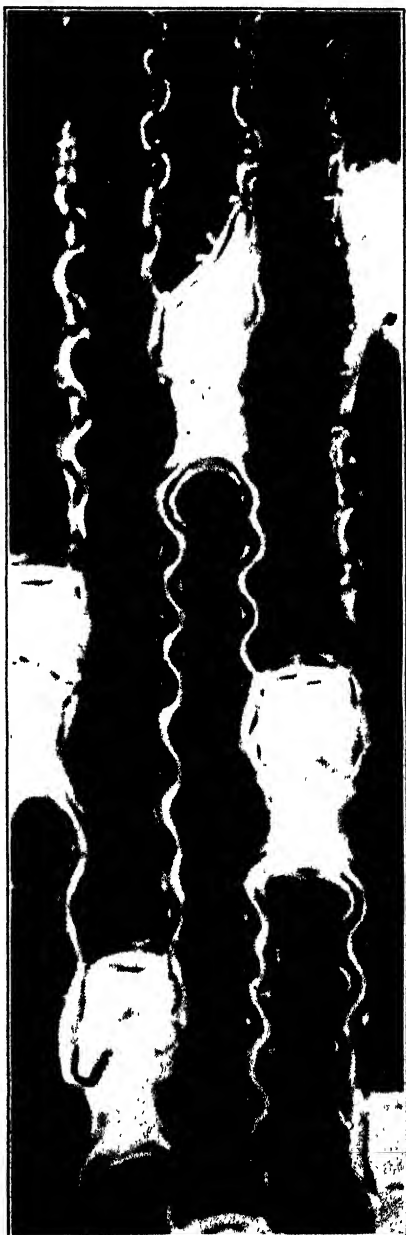


FIGURE 1.—Epidermis of Kassoei $\times 1,000$

hairs (fig. 4). These elongated cells are placed by Mameli de Calvino⁷ in a special class; they are, however, only an extreme type of cork cell. The walls usually stain with chloriodide of zinc a golden yellow, while the lumen often contains silica. Sometimes the walls of these elongated cork cells are very thick and heavily infiltrated with silica so that they appear a glistening white in the preparation. The cork cell of a group in whatever form it occurs always lies vertically above the silica cell.

The silica cells are very uniform and usually rectangular with the long diameter parallel to the long axis of the stem. (Pl. 1, A, sc.) Occasionally they are broader than long, oval, or even round. In surface view they appear biscuitlike with slightly overhanging margins. The cells are practically solid, though in the center one commonly observes small air spaces often arranged in a row and containing some refractory material, the remains of the protoplast.

Comparatively few stomata are found in the epidermis of the stem, though there are varietal differences of which significant use can

be made in classification.

The structure of the stomata is normal except that its cells become strongly silicified, remaining pure white in the preparation.

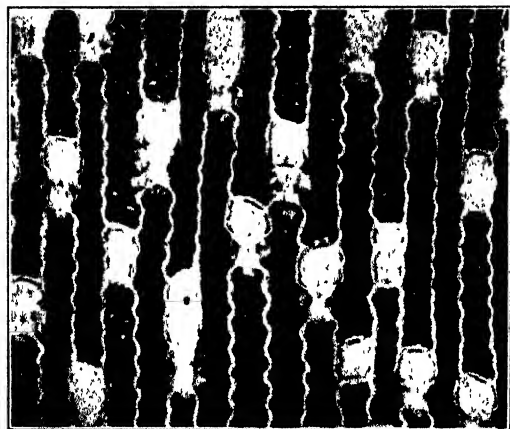


FIGURE 2.—Epidermis of Cayana. $\times 500$

THE EPIDERMIS OF CERTAIN VARIETIES

D 74

The variety D-74 stands more or less by itself because of the small size of its epidermal cells. (Fig. 5.) There are between 90 and 130 rows of long cells in a square millimeter, so that the average width of the cells

varies from 7.7μ to 11.1μ . The number of short-cell groups in a square millimeter reaches 1,112 or more. Because of the large number of short cells, the long cells also are relatively short. However, the variation in length is so great that the determination of a mean value would have little significance. The short cells in section appear square, rectangular, or trapezoidal, and occasionally pointed. Sometimes they occur in pairs with one member of the pair a cork cell, the other a silica cell; sometimes the cork cell stands alone. The number of stomata varies, but as many as 14 have been counted in a microscopic field covered by a 16-mm. objective and a No. $\times 6$ ocular, or an area of 2.4 sq. mm.

LOUISIANA PURPLE

In the Louisiana Purple variety also there is a preponderance of short cells (fig. 3), for as many as 928 were counted in a square millimeter, and, since they are comparatively large, their total area equals that of the long cells and may even exceed it. This fact becomes

⁷ Op. cit., p. 179. (See footnote 4.)

evident at once if one views the epidermis under low magnification. There are, on an average, 100 rows of long cells in a square millimeter, so that the average cell width is about $10\ \mu$. The long cells are, on the whole, comparatively short and their end walls are usually transverse. The cork cells are reniform, square, or pointed, but hairlike cork cells are rare. The short cells appear most often in pairs or double pairs with one member a cork cell, the other a silica cell. The number of stomata varies with the microscopic field. At most, however, there are only one or two stomata in a field.

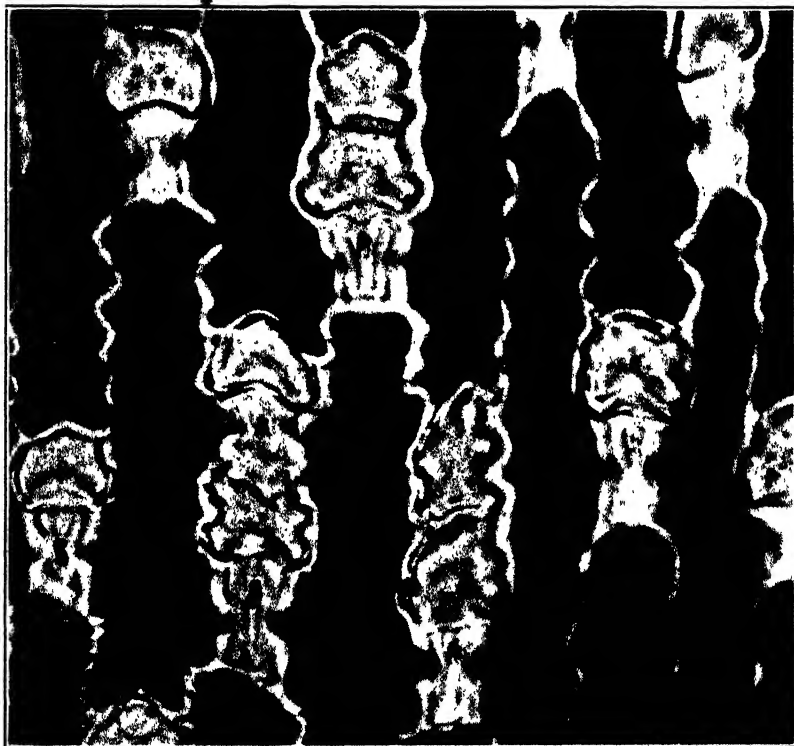


FIGURE 3.—Epidermis of Louisiana Purple. $\times 1,000$

YELLOW CALEDONIA

The Yellow Caledonia variety is characterized by the partial or nearly complete absence of silica cells, a fact which makes it very easy to recognize, especially if one takes other characteristics into consideration. The pattern of the epidermis, viewed under low power, seems to vary. Sometimes the long cells are very regular in length and width; again the cells are variously shaped. Commonly, however, the long cells are rather narrow, there being 110 to 120 rows in a square millimeter, so that the width of the individual cell is on an average only 8.3 to $9\ \mu$. The number of short-cell groups is about 800 in a square millimeter; the cells are trapezoidal, often pointed and hairlike. The cork cells are found singly or in groups, sometimes in pairs

with one member a silica cell. In general, however, silica cells are few in number. With low power one notices longitudinally disposed rows of epidermal cells which possess thicker and more strongly silicified walls than the neighboring rows. The lumen of the cells often contains silica. Stomata are also quite rare, since never more than one is observed in a microscopic field.

P. O. J. 2714

The pattern of the epidermis of P. O. J. 2714 is very uniform and the cells themselves are regular. There are about 94 rows of long cells in a square millimeter, so that their average width is $10.6\ \mu$. The number of short-cell groups is 816 to 916, which closely agrees with the previously described varieties. The long cells are apt to vary considerably in width. Since their walls are of medium thickness or less, these cells have a comparatively broad lumen and appear to be wider than they actually are.



FIGURE 4.—Epidermis of P. O. J. 213.
× 330

A comparatively large number of the long cells are filled with crystal sand. The short-cell groups contain in most cases only one type of cell, the cork cell. Because of the absence of the silica cell, the cork cell very often stands alone; occasionally the cork cells appear in twos and even threes. When solitary the cork cell is rectangular or trapezoidal; in association with other cork cells it is shorter and sometimes pointed. Stomata are rather common, although their distribution is erratic. One often counts as many as 10 or more in a microscopic field.

U. S. 759

U. S. 759, which is relatively thin stalked as compared with the varieties described above, possesses an epidermis made up of comparatively large cells. The epidermal pattern is remarkably uniform. There are 72 rows of long cells in a square millimeter, so that their average width is $13.6\ \mu$. The group of short cells is also greatly reduced in number, there being only 428 to 440 in a square millimeter as contrasted with 1,000 or more in D-74. The long cells are very uniform and possess transverse end walls. The calcium oxalate cells occur singly or in groups and are readily recognized by their glistening contents. The composition of the short-cell groups is similar to that in the preceding variety. The short cells appear in pairs (fig. 6) or the silica cell is wanting. The distribution of the stomata is erratic. Sometimes two are counted in a microscopic field; sometimes not even one.

P. O. J. 36

The P. O. J. seedlings that have Chunnee as the male parent are characterized by an epidermis in which the elongated or pointed cork cells are conspicuous and in which the epidermal pattern is more or less irregular.

The epidermis of P. O. J. 36 is quite irregular. The long cells appear narrow, more or less curved, and the end walls are often pointed. There are 74 rows of long cells in a square millimeter, their average width being 13.54 μ . The number of short-cell groups varies greatly and is difficult to estimate. The cork cells are quite irregular, often pointed and hairlike. The distribution of stomata is as irregular as in the other varieties. There are on an average 5 in a microscopic field, though as many as 12 have been counted.

P. O. J. 213

The epidermis of P. O. J. 213 is commonly very irregular and contains many elongated or hairlike cork cells. (Fig. 4.) The long cells have oblique or transverse end walls and are often of considerable length. They appear quite narrow, but actual counts show that there are on an average 89 rows of long cells in a square millimeter, so that their average width is 11.25 μ . The short cells appear in groups of 10 or more, one member of the group a silica cell. While the silica cells have the normal rectangular form characteristic of all varieties, the cork cells are variously shaped; and, since many of them are considerably elongated, the entire area they cover is at least equal to that covered by the ordinary epidermal or long cells. The actual number of short-cell groups in a square millimeter is approximately 372. Stomata are rare, usually less than one in a microscopic field.

P. O. J. 234

Compared to the variety just described, which it resembles greatly, P. O. J. 234 possesses a much more regular epidermis. The long cells are uniform and commonly have rectangular cross walls. There are 80 rows in a square millimeter, so that the average width of the cell

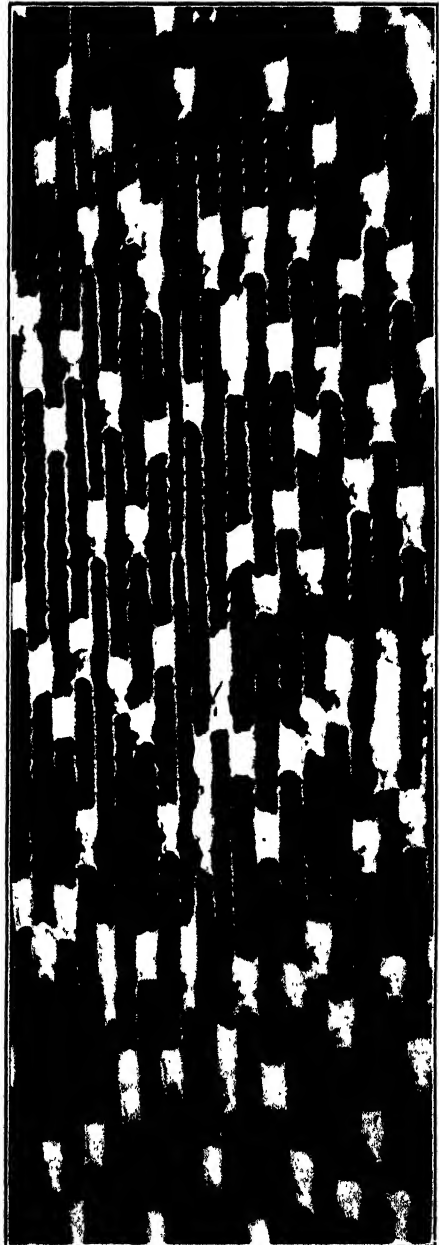


FIGURE 5.—Epidermis of D-74. $\times 330$

is $2.5\ \mu$. The number of short-cell groups is greater than in the former variety, there being between 376 and 484 in a square millimeter. The groups of short cells are made up of both cork cells and silica cells. The cork cells, though of somewhat variable form, are much less conspicuous than those of P. O. J. 213. The cells are more or less rectangular, though there is a tendency for them to be triangular and even hairlike. Stomata, however, are more abundant, there being on an average four in a microscopic field.

KASSOER

The epidermal pattern in the Kassoer variety also is quite regular. (Fig. 7.) The long cells are relatively short, but sometimes much

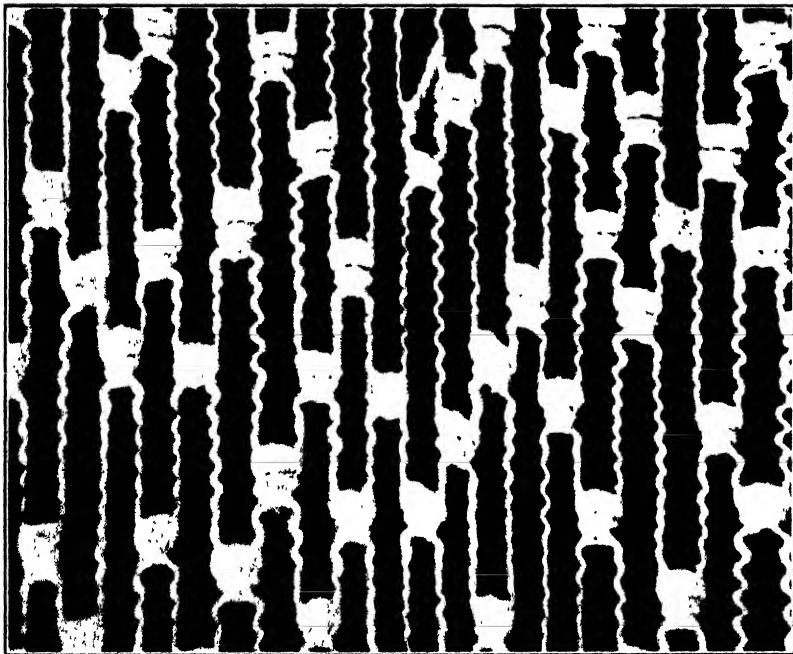


FIGURE 6.—Epidermis of U. S. 759. $\times 330$

elongated; their end walls are typically rectangular but occasionally pointed. There are on an average 75 rows of long cells in a square millimeter, so that the average width of the long cells is $3.3\ \mu$. The short cells are in groups of two or more, but the silica cell is occasionally wanting, so that the cork cells are solitary. Occasionally a silica cell stands alone between the long epidermal cells. The number of short-cell groups in a square millimeter varies from 364 to 480. The shape of the cork cell is commonly broadly reniform; occasionally the cells are rectangular, more often pointed and hairlike. The distribution of stomata is erratic, there being on an average one or two in a microscopic field.

U. S. 663

The epidermis of variety U. S. 663 appears very regular. (Fig. 8.) The long cells are uniform and have straight, sometimes pointed end

walls. There are 80 rows of long cells in a square millimeter, so that their average width is $12.5\ \mu$. The number of short-cell groups varies, there being from 480 to 540 in a square millimeter. The cork cells are reniform, sometimes rectangular, sometimes pointed, and occasionally hair-like. The short cells occur in pairs; often, however, the silica cell is wanting. There is commonly one but sometimes as many as five stomata in a microscopic field.

U. S. 833

The epidermis of U. S. 833 resembles that of U. S. 663, except that the long cells are relatively short. The pattern is very uniform. There are 75 rows of long cells in a square millimeter, so that their average width is $13.3\ \mu$. The number of short-cell groups is very uniform, there being on an average 656 in a square millimeter. The cork cells are usually reniform, occasionally square or pointed, sometimes hairlike. The short cells occur in pairs with one member a cork cell, the other a silica cell. Stomata are numerous, with as many as 15 in a microscopic field.

CAYANA

The pattern of Cayana is fairly uniform. (Fig. 2.) The long cells appear comparatively broad and short, but often they are quite elongated. Their end walls are usually rectangular. There are 75 rows of long cells in a square millimeter, so that their average width is $13.6\ \mu$. The number of short-cell groups averages 644 in a square millimeter. The cork cells are uniform, sometimes rectangular, sometimes pointed. They are usually joined to silica cells, but the latter may occasionally be wanting so that the cork cells stand alone. Stomata are rare, but occasionally four to six are counted in a microscopic field.

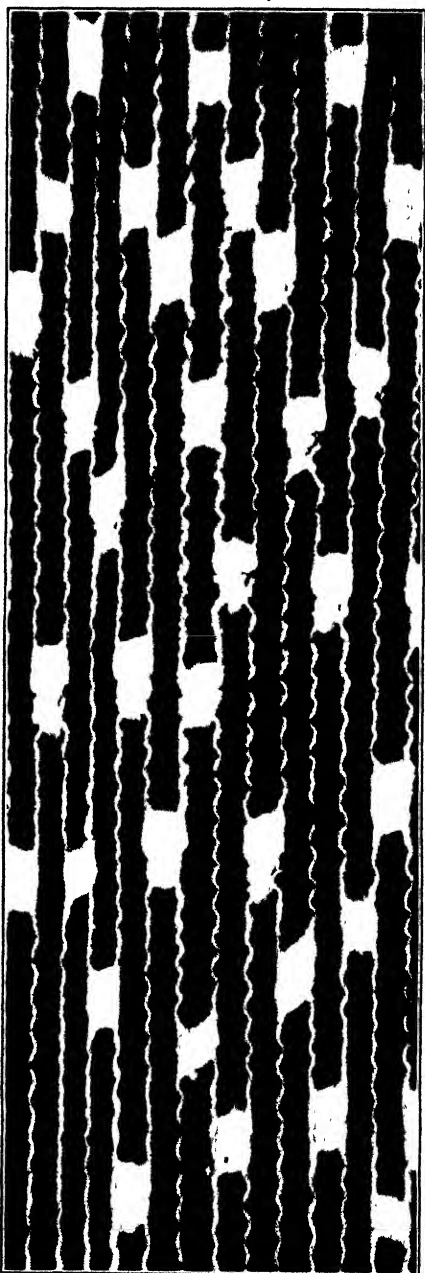


FIGURE 7.—Epidermis of Kassoer. $\times 330$

CHUNNEE

The epidermis of the Chunnee variety is composed of small cells with an average diameter of $7.6\ \mu$. It has, however, only 504 groups of small cells in a square millimeter. It differs in this respect from variety D-74, which, besides having narrow epidermal cells, has also a large number (over 1,000) of groups of small cells. The pattern of the epidermis is very uniform, with silica and cork cells in typical pairs. Stomata are infrequent.

SACCHARUM SPONTANEUM

Although *Saccharum spontaneum*, like Chunnee, is a thin and fibrous cane, the epidermis is made up of large cells (fig. 9, A) with an average width of $12.5\ \mu$. The number of short-cell groups is small, rarely exceeding 400 square millimeters. Silica cells are usually wanting, but stomata (fig. 9, B) are comparatively abundant.

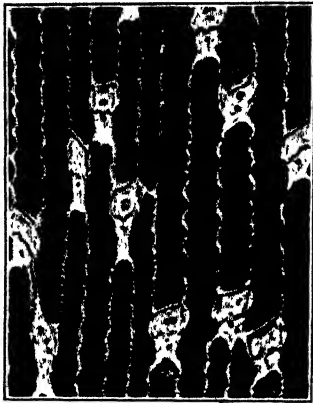


FIGURE 8 - Epidermis of *S. S. 663*.
X 500

DISCUSSION

The variations in epidermal structure, as shown in Table 1, fall into two classes—qualitative and quantitative. Qualitative differences consist in the absence of silica cells and of elongated or hair-like cork cells. In the last analysis, however, these also are quantitative variations, since there is rarely a complete absence of either type; but even the partial absence of silica cells presents a picture so striking that some varieties could be recognized by this character alone. This is true, to a lesser degree, of the elongated or pointed cork cells also. There are varieties in which they are practically absent, while in others, especially P. O. J. 213 and 36, they are so abundant that one would experience little difficulty in identifying the variety. Among quantitative variations are the width and length of the long epidermal cells, the number of short-cell groups in a unit area, and the distribution of the stomata.

The variation in the width of the epidermal cells often aids in the separation of varieties when other diagnostic characters fail. It is important, however, to estimate correctly the average width of the cells. This may be difficult in varieties which have an irregular epidermis, as for example, P. O. J. 36. One can calculate the average width of the long cells by counting the number of rows in the microscopic field and dividing the diameter of the field by that number; more accurate counts, however, are obtained by projecting the stained preparation on a white screen. The thickness of the walls of the epidermal cells is more or less uniform. As a rule, a narrow-celled epidermis, as for example in D-74, has comparatively thin walls. The type of undulation of the walls also varies in the different varieties, as can easily be seen in the accompanying figures.

A certain ratio exists between long-cell and short-cell areas which can be calculated by determining the number of groups of short cells

in a unit area. It must be borne in mind, however, that in varieties with single-cell groups, that is, where the silica cells are wanting, the area of short cells will be less, while in varieties with a large number of elongated or hairlike cork cells this area will be greater than the calculated value.

The distribution of stomata, although erratic, offers a valuable diagnostic character. P. O. J. 213 and P. O. J. 234 are much alike in

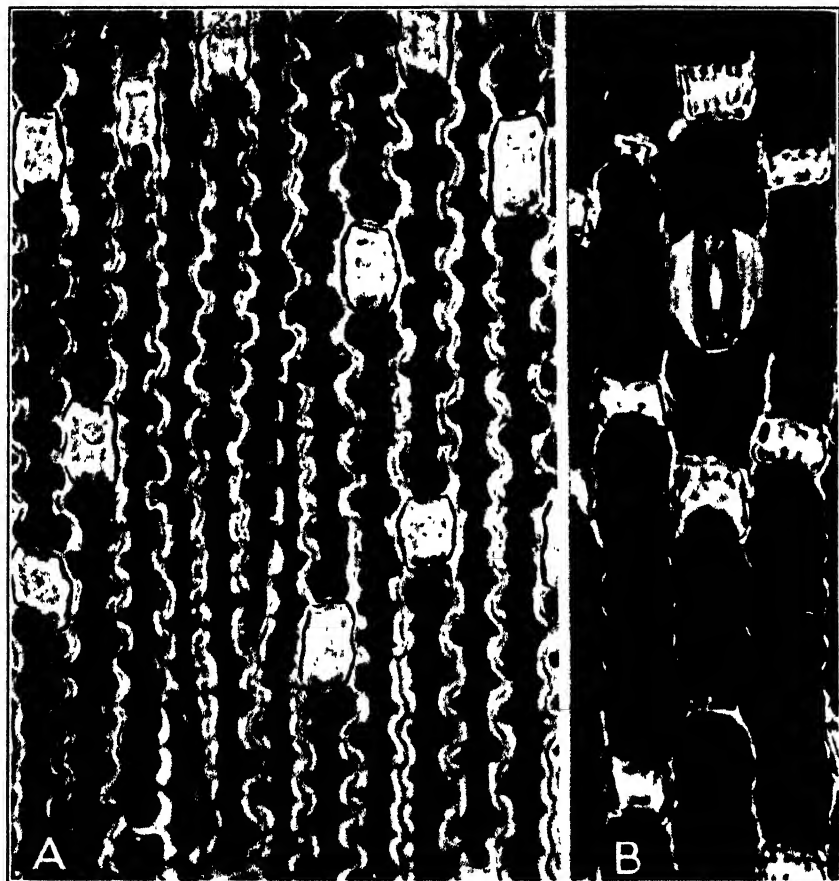


FIGURE 9 - Epidermis of *Saccharum spontaneum*: A, Showing solitary cork cells. $\times 500$. B, Showing a stoma and pitting of the cork cells $\times 1,000$

the appearance of the epidermis; the former, however, has only one or two stomata per microscopic field, while the latter has from four to six.

In order to trace group relationships and analyze the parental influence in the various crosses, it would be necessary to examine the epidermis of a large number of canes, especially the early parent types. It would be of interest to trace the development of the elongated or hairlike cork cells back to the ancestral forms. All P. O. J. canes examined, with the exception of P. O. J. 2714, possess this type of cell. The male parent of these varieties is Chunnee and the female parents are Louisiana Purple and Gestrept Preanger, respectively. Neither

of the parents possesses the elongated cork cells, and it might be inferred that this character belongs to some earlier ancestor. In Kassoer and the U. S. seedlings these pointed cork cells are practically absent. At the same time these varieties exhibit in a greater or less degree a suppression of the silica cells, causing many of the short-cell groups to be composed of solitary cork cells. These two characters—that is, absence of elongated cork cells and suppression of the silica cells—are most significant in the epidermis of *Saccharum spontaneum*, a distant male ancestor of these varieties. The distribution of stomata is another character that defies a ready analysis. There appears to be no correlation between the number of stomata and the size of the epidermal cells. In D-74, which is the smallest-celled variety studied, the number of stomata is comparatively large, while in Chunnec, which also possesses small cells, stomata are rare.

TABLE 1 -- *Epidermal characters useful in separating cane varieties*

Variety	Average width of cells μ	Number of short-cell groups per square millimeter	Pointed elongated cork cells	Solitary cork cells	Number of stomata per microscopic field ^a
Louisiana Purple	10.0	± 928	Absent	Scarce	1-2
D-74	7.7	$\pm 1,112$	do	Abundant	14
Yellow Caledonia	9.1	896-780	Present	do	1 or less
P. O. J.					
2714	10.6	816-916	Absent	do	10
36	13.54	± 528	Present	Absent	12
213	11.25	± 372	do	do	1
234	12.5	376-484	do	do	4
Kassoer	13.3	364-480	Scarce	Scarce	1-2
U. S.					
603	12.5	480-540	Absent	Abundant	1
833	13.3	± 656	do	Absent	15
759	13.6	428-440	do	Abundant in places	2
Cayana	13.6	± 644	do	Scarce	4-6
<i>Saccharum spontaneum</i>	12.5	± 400	do	Abundant	---
Chunnec	7.6	± 504	do	Absent	---

^a Covered by a 16-mm objective and a No. X6 ocular, or an area of 2.4 sq. mm.

The following key affords, in a restricted sense, a means of identifying sugarcane varieties and hybrids of *Saccharum officinarum* and of tracing group relationships. It is as yet limited in its scope because of the restricted number of varieties studied, but its field of usefulness may be increased by extending the investigation to all important varieties.

KEY

1. Epidermis small-celled. Number of short-cell groups exceeding 20 per microscopic field (2-mm. oil-immersion objective and No. 6 eyepiece covering an area of 0.025 sq. mm.). Average width of elongated epidermal cells mostly below 10 μ .
 1. Elongated pointed cork cells numerous. *Yellow Caledonia*.
 2. Elongated cork cells wanting or few in number.
 - A. Cork cells often solitary. *P. O. J.* 2714.
 - B. Short-cell groups usually in pairs or double pairs with one member of the pair a cork cell, the other a silica cell.
 - a. Stomata numerous. *D-74*.
 - b. Stomata scarce. *Louisiana Purple*.

11. Epidermis large celled. Number of short-cell groups 16 or less per microscopic field
1. Elongated cork cells more or less conspicuous.
 - A. Stomata rare. *P. O. J. 213.*
 - B Stomata numerous.
 - a. Elongated cork cells abundant; 5-12 stomata per field (16-mm. objective, No. 6 eyepiece) *P. O. J. 36.*
 - b. Elongated cork cells less conspicuous; stomata 4-6 per field *P. O. J. 234.*
 2. Elongated cork cells few in number or wanting.
 - A. Stomata scarce, rarely more than one per microscopic field.
 - a. Solitary cork cells numerous. *U. S. 663*
 - b. Solitary cork cells less evident.
 - (1) Distribution regular *Kassner.*
 - (2) Distribution erratic *U. S. 759.*
 - B Stomata numerous *U. S. 833.*

SUMMARY

The stem epidermis of sugarcane can be removed for study by the use of potassium chlorate and nitric acid, and its component structures differentiated by staining with chloroiodide of zinc.

The epidermis is composed of ordinary or "long" cells and of "short" cells. The latter occur commonly in pairs with one member a cork cell, the other a silica cell. The silica cell is biscuit-shaped, varies little in size and form, and may be wanting. The cork cell is reniform, rectangular, trapezoidal, or elongated like a hair. The occurrence of elongated cork cells is more or less restricted to certain varieties. Stomata are very rare in some varieties, less so in others, and always erratic in distribution.

Since the structure of the epidermis is characteristic of the variety and since its composition appears to be little influenced by environmental factors, it is useful in identifying varieties.

CHROMOSOME BEHAVIOR AND POLLEN PRODUCTION IN THE POTATO¹

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INTRODUCTION

The propagation of potatoes from seeds is seldom resorted to by the commercial grower, asexual reproduction by means of tuber cuttings being the universal practice. Seeds, however, have been much used by potato breeders as a means of producing new varieties because of the wide range of variability which has been found to occur in the progenies produced in this manner.

It is generally known that seed production in the potato is the exception rather than the rule. Numerous investigations have been made to determine the reason for the nonfruitfulness of potatoes. East (4),² Dorsey (3), and more recently Breeze (1) found that there is a great variation in the amount of viable pollen produced by different varieties of potato and that in many of them there is a large percentage of defective pollen. Stout and Clark (18) made an extensive study of pollen conditions both in wild potato species and in cultivated varieties. In the wild species they generally found abundant viable pollen, while among cultivated varieties they found very few that produced an appreciable amount.

Pollen studies show the plant breeder that the lack of viable pollen is largely responsible for the nonproduction of seed in potatoes, but he is still in the dark as to the cause of the high percentage of abortive pollen. For this reason the writers undertook to investigate the pollen mother-cell development of the cultivated potatoes and their allies. It was hoped that such a study would (1) give a more complete picture of the sterility problems that confront the potato breeder, and possibly indicate why cultivated potatoes differ so markedly from wild species in their ability to produce viable pollen; and (2) show whether varietal differences are associated with chromosomal differences and whether anything in the number or behavior of the chromosomes would give a clue to the ancestry of the cultivated potato. It was believed that information on these points might be useful to potato breeders.

MATERIAL AND METHODS

This study treats of the number and meiotic behavior of chromosomes in tuber-bearing forms of *Solanum*. These forms came originally from widely different sources and belong to the section *Tuberarium* of the genus *Solanum*. Cytological material was collected from 2 unnamed seedlings and 38 commercial varieties belonging to the group known as *Solanum tuberosum* L. and from 11 wild forms referred to 10 distinct species.

¹ Received for publication July 24, 1930; issued December, 1930.

² Reference is made by number (italic) to Literature Cited, p. 887

The buds from which material showing the developing pollen mother cells was taken were collected, with few exceptions, at the Aroostook Farm of the Maine Agricultural Experiment Station at Presque Isle, Me., in the plots grown under the supervision of the Office of Horticultural Crops and Diseases of the Bureau of Plant Industry, United States Department of Agriculture.

Material was collected during the latter part of July in the years 1928 and 1929. The collections were made during the warmer part of the day, and acetocarmine preparations were made at once from fresh material. Other material was killed in Carnoy's fluid and brought to the laboratory at Washington for future study.

The preparations in acetocarmine made from fresh material were most satisfactory for studying chromosome numbers. The killed and fixed material was useful, however, in studying the general type of divisions and the character of the tetrads typical of the different varieties.

The collecting of material in two different seasons made it possible to compare the behavior of a variety grown in slightly different environments.

Camera lucida drawings and photomicrographs were made of characteristic figures observed in the preparations. A Leitz $\frac{1}{16}$ apochromatic oil-immersion lens and $\times 8$ and $\times 15$ oculars were used throughout these studies.

CHROMOSOMES IN WILD SPECIES OF SOLANUM

Plants are frequently found in which the somatic chromosome complement is made up of more than two intermingled sets. Such plants are spoken of as being polyploid. The term "polyploid" is not restricted to plants known to have more than two homologous chromosome sets, but is frequently applied to forms in which the chromosome number alone is the only suggestion of the presence of several sets of chromosomes. It is in this sense that the present writers use the terms "triploid," "tetraploid," etc.

Eleven wild *Solanum* forms shown in Table 1 have their diploid chromosome numbers in definite multiples of 12. Since no *Solanum* species has been found with less than 12 as its haploid or less than 24 as its diploid chromosome number, these are considered the basic chromosome numbers for the genus, and the species with 36, 48, and 72 somatic chromosomes are spoken of as being triploid, tetraploid, and hexaploid, respectively.

TABLE 1. - *Chromosome numbers in wild forms of potato*

Name	Chromosome class	Haploid chromosome number	Investigator
<i>Solanum caldasii</i> ...	Diploid...	12	Vilmorin and Simonet (20, 21)
<i>Solanum caldasii glabrescens</i> ...	do	12	Present writers.
<i>Solanum chacoense</i> ...	do	12	Smith (17), present writers.
<i>Solanum jamesii</i> ...	do.	12	Vilmorin and Simonet (20, 21), Smith (17); present writers
<i>Solanum polyadenium</i> ...	do	12	Present writers.
<i>Solanum cardiophyllum</i> f. <i>corymbosum</i> ...	Triploid	18	Do.
<i>Solanum commersonii</i> ...	do	18	Do.
<i>Solanum ajacense</i> ...	Tetraploid	24	Do.
<i>Solanum antipovichi</i> ...	do	24	Do.
<i>Solanum fendleri</i> ...	do.	24	Smith (17); present writers.
<i>Solanum demissum</i> ...	Hexaploid...	36	Do.

Chromosome counting is frequently a difficult problem. For this reason gametic cells, in which the reduced or haploid chromosome number is found, are most often used by cytological investigators. The present study of chromosome number has been for the most part confined to the developing pollen mother cells.

The four wild forms *Solanum caldasii glabrescens* Dunal, *S. chacoense* Bitter, *S. jamesii* Torr., and *S. polyadenium* Greenm. have 12 as their haploid chromosome number. A and B of Figure 1 show first-division metaphases of *S. caldasii glabrescens* and *S. polyadenium*, respectively. A second-division metaphase of *S. chacoense* is illustrated in Figure 1, C. All four species show regular pairing and distribution of chromosomes during the meiotic phases of their pollen mother-cell development.

The chromosome behavior during the reduction divisions of F_1 and F_2 hybrids between the two species *Solanum caldasii glabrescens* and *S. chacoense* was studied. In both generations there was a regular pairing of the chromosomes, and a large percentage of the chromosome

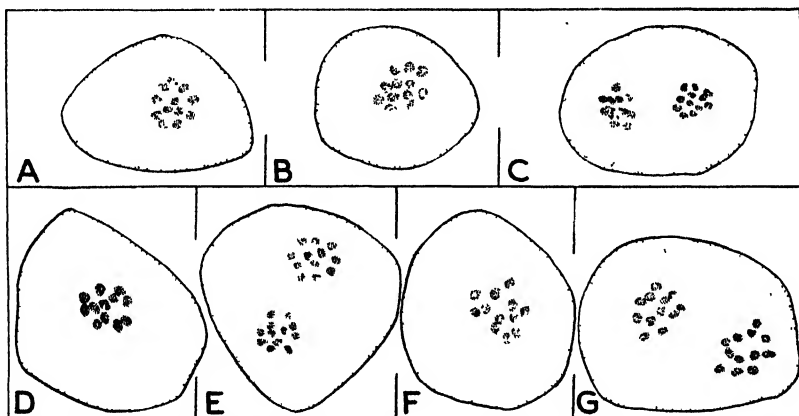


FIGURE 1.—Pollen mother cells from diploid species and hybrids. A, B, D, and F, First-division metaphase in *Solanum caldasii glabrescens*, *S. polyadenium*, *S. tuberosum* S. A. 336, and *S. caldasii glabrescens* \times *S. chacoense*, respectively. C, E, and G, Second-division metaphases of *S. chacoense*, *S. tuberosum* S. A. 336, and *S. caldasii glabrescens* \times *S. chacoense*, respectively. $\times 2,000$

tetrads were normal in appearance. Figure 1, F, shows a first-division metaphase from an F_1 hybrid, in which 12 bivalent chromosomes are arranged on the plate. Figure 1, G, is a second-division metaphase from an F_2 hybrid; in both plates the 12 chromosomes are clearly shown.

Two wild forms, *Solanum cardiophyllum* f. *coyoacanum* Bukasov and *S. commersonii* Dunal, were found to be triploid, with 18 as their haploid chromosome number. In any preparation from either of these species there were very few cells that showed a regular pairing of all chromosome units. The number of paired chromosomes varied from 12 to 18, with a corresponding variation in the number of univalents. Figure 2, A, shows a first-division metaphase of *S. cardiophyllum* f. *coyoacanum* with 8 univalents and 14 bivalents; B shows a cell of *S. commersonii* with 4 univalents and 16 bivalents; and C is a second-division metaphase of the latter species showing unequal numbers of chromosomes in the two plates and two extruded chromosomes.

The chromosome behavior of both of these forms is that usually resulting when two sets of incompatible chromosomes, or when two unequal chromosome sets, attempt to pair in the early phases of the first division of a hybrid plant. It is almost identical with the meiotic behavior of the artificially produced triploid hybrid described in the following paragraph.

Material from an F_1 hybrid between *Solanum fendleri* with 24 chromosomes and *S. chacoense* with 12 gave a chromosome behavior typical of a plant hybrid combining two unequal sets of chromosomes. Figure 2, D and E, are two first-division metaphases showing both paired and unpaired chromosomes. The bivalent chromosomes behave normally, but the univalents are distributed undivided at random in the first division, and frequently a few fail to be included in the two daughter nuclei. Figure 2, F, shows a second-division metaphase with 18 chromosomes on each plate. Many cells, however,

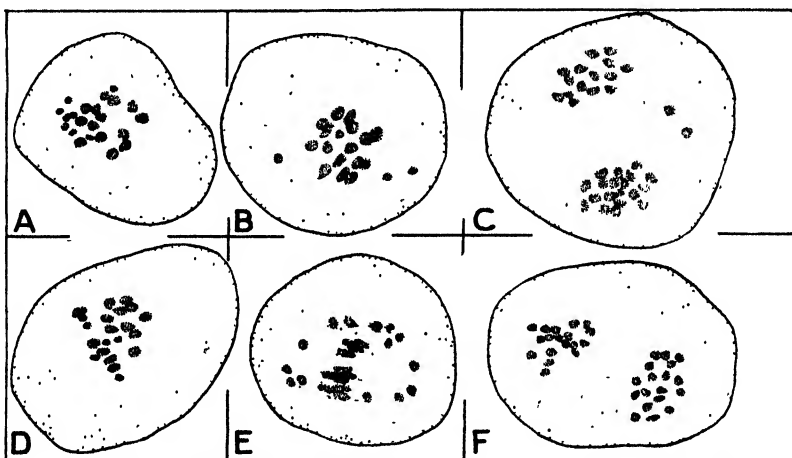


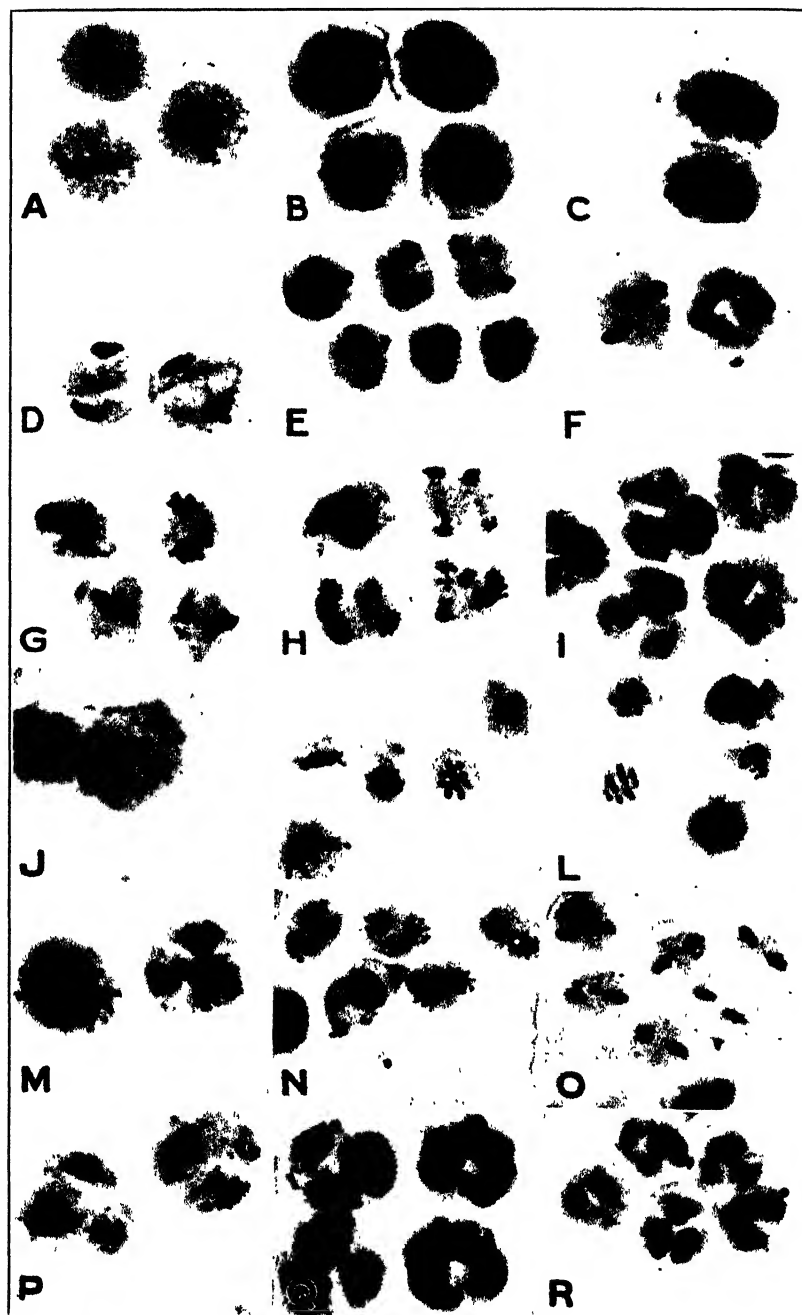
FIGURE 2.—Pollen mother cells from triploid forms and hybrids. A and B, First-division metaphases in *Solanum cardiophyllum* f. *cozacacum* and *S. commersonii*, respectively. D and E, First-division metaphases in *S. fendleri* \times *S. chacoense*, face view and side view, respectively. F and G, Second-division metaphases in *S. commersonii*, showing two chromosomes in the cytoplasm, and *S. fendleri* \times *S. chacoense*, respectively. $\times 2,000$

at this phase show unequal numbers on the two plates, and later phases frequently show smaller chromosome groups in addition to the four major groups.

Three wild species, *Solanum fendleri* Gray, *S. ajuscoense* Bukasov, and *S. antipovichi* Bukasov, were found to have twice as many chromosomes as the diploid species. A, B, and D of Figure 3 show first-division

EXPLANATORY LEGEND FOR PLATE 1

- A and B.—First-division metaphases in Prince Albert and seedling No. 43752, respectively.
 C and D.—Interkineses in Keeper and Prince Albert, respectively.
 E.—Second-division metaphases in Keeper.
 F and G.—Second-division metaphases in McCormick.
 H.—Second-division anaphases in Keeper.
 I.—Tetrads in seedling No. 43752.
 J, K, and L.—First-division metaphases in *Solanum demissum*, *S. tuberosum* S. A. 302, and *S. caldasii* *glabrescens* \times *S. chacoense*, respectively.
 M.—First-division anaphase in *S. antipovichi*.
 N and O.—Second-division metaphases in *S. caldasii glabrescens* \times *S. chacoense* and *S. tuberosum* S. A. 302, respectively.
 P, Q, and R.—Tetrads in *S. antipovichi*, *S. demissum*, and *S. tuberosum* S. A. 302, respectively.



metaphases of these three species, in which 24 chromosomes may be counted. There was very little difficulty in obtaining good figures of both the reduction divisions in all three species, although *S. ajuscoense* showed considerable irregularity. Figure 3, C, illustrates a regular second-division metaphase of *S. ajuscoense*; the lower plate shows a side view, the upper plate a face view; in the latter the 24 chromosomes stand out distinctly.

Only one species, *Solanum demissum* Lindl., was found to have 36 as its haploid chromosome number. Figure 3, E, shows a first-division metaphase of a pollen mother cell from this form, in which the number is clearly distinguishable. The meiotic behavior of the chromosomes is regular and leads to the production of normal pollen tetrads. Smith (17) has found the same number for this species and reports nothing abnormal in its chromosome behavior.

Plate 1, J-R, is a series of photomicrographs illustrating the character of the first and second reduction-division phases in a group of regular-behaving potatoes, most of which are from the foregoing wild

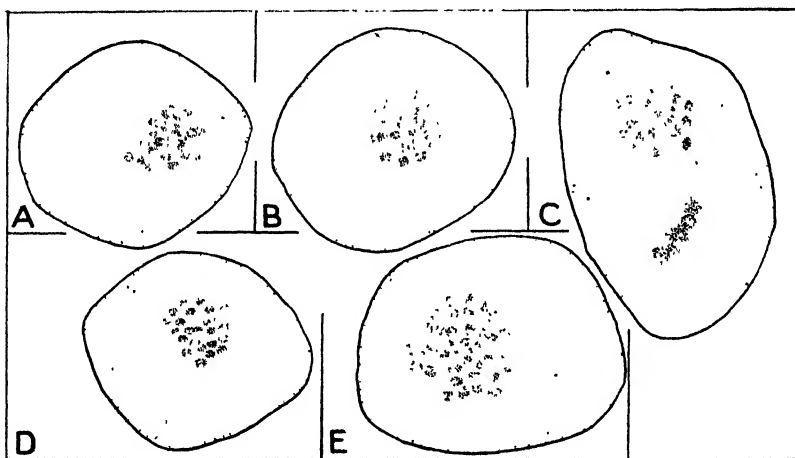


FIGURE 3.—Pollen mother cells from tetraploid and hexaploid species. A, B, D, and E, First-division metaphases in *Solanum antiporichi*, *S. ajuscoense*, *S. fendleri*, and *S. demissum*, respectively. C, Second-division metaphase in *S. ajuscoense*. $\times 2,000$

species. J is a side view of a first-division metaphase of *Solanum demissum* in which the chromosomes are lying uniformly on the plate; L shows both face and side views of first-division metaphases of *S. caldasii glabrescens* \times *S. chacoense* in which the chromosome pairing is regular. M shows a first-division anaphase and a 4-celled tetrad of *S. antiporichi*. N shows second-division metaphases and anaphases of *S. caldasii glabrescens* \times *S. chacoense* which give no indication of irregularities in the second division. P and Q show tetrads of *S. antiporichi* and *S. demissum*, respectively. The chromosome behavior of any of these species is seldom irregular, and the tetrads usually appear normal.

This study of chromosomes in wild species of *Solanum* has shown that forms with an even multiple of the basic chromosome number, except possibly *S. ajuscoense*, have a regular meiotic behavior resulting in the production of 4-celled tetrads and that each cell of the

terad receives the same number of chromosomes. The chromosome behavior in the two triploid forms, however, suggests that they are hybrids in which two unequal chromosome sets or three chromosome sets have been brought together. The presence of univalent chromosomes results in an abnormal distribution of chromosomes to the daughter cells during pollen formation. The chromosome behavior of the two triploid forms is very similar to that described for the *S. fendleri* × *S. chacoense* hybrid, in which the chromosome sets of the two parents were unequal (possibly the female parent had two sets and the male one), and causes abnormal meioses typical of a hybrid of this type.

The second hybrid, *Solanum caldasii glabrescens* × *S. chacoense*, in which two equal chromosome sets were combined, showed nothing of an incompatible nature when the chromosomes of the two distinct species were brought together during meioses of the developing pollen mother cells.

CHROMOSOMES IN CULTIVATED POTATOES

Determinations of the chromosome number in 40 cultivated forms (Table 2) of *Solanum tuberosum* have shown that all except three yellow-fleshed varieties from South America have 24 as their gametic chromosome number. A study of the meiotic phases of these tetraploid varieties, however, show that very few of them have a regular chromosome behavior during pollen formation.

TABLE 2.—Cell characteristics associated with pollen sterility in cultivated potatoes (*Solanum tuberosum*)—Continued

Year	Field No.	Variety	Distribution of pollen mother cells producing the following groups of cells—						4-cell groups		Pollen classes ^a	Chromosomes	
			2	3	4	5	6 or more	Total	Percentage	Standard error		Behavior	Number
1928	53	Dakota Red.	97	6	1	—	—	97	1.03	.939	4	Group 3.	24
1929	29	do	106	7	—	—	—	113	0	—	4	do.	24
1928	11	Early Mauntee	92	9	3	—	—	104	2.88	1.67	4	do.	24
1929	17	do	139	19	4	—	—	162	2.47	1.23	4	do.	24
1929	11	Early Ohio.	120	9	—	—	—	129	0	—	3	do.	24
1929	A	Early Rose.	107	8	—	—	—	115	0	—	3	do.	24
1928	56	Jersey Red Skin.	100	7	—	—	—	107	0	—	3	do.	24
1929	—	do	129	17	—	—	—	146	0	—	3	do.	24
1929	4	Noroton Beauty.	67	17	—	—	—	84	0	—	3	do.	24
1929	—	Peoples.	102	—	2	—	—	104	1.92	1.31	—3	do.	24
1929	87	Peerless.	117	2	—	—	—	119	0	—	—	do.	24
1929	—	Peerless (Pearl).	115	—	1	—	—	116	0	.87	3	do.	24
1929	31	Queen of the Valley.	133	16	—	—	—	149	0	.86	—3	do.	24
1928	41	Russet Rural.	55	26	2	—	—	83	2.41	1.68	3	do.	24
1929	24	do	100	9	—	—	—	109	0	—	3	do.	24
1928	9	Scotch Rose.	107	28	—	—	—	135	0	—	3	do.	24
1929	—	do	86	17	3	—	—	106	2.83	1.60	3	do.	24
1929	—	Triumph.	78	19	3	—	—	102	4.90	2.14	3	Group 3.	24
1928	34	Adirondack.	105	22	—	—	—	127	0	—	3	Group 4.	24
1929	30	do	63	1	—	—	—	64	1.30	1.20	3	do.	24
1928	61	Charles Downing.	86	14	5	—	—	105	4.76	2.10	4	do.	24
1929	—	do	91	8	2	—	—	102	1.96	1.37	4	do.	24
1929	1	do	61	3	2	—	—	66	3.03	2.09	4	do.	24
1929	39	Cowhorn.	105	—	—	—	—	105	0	—	4	do.	24
1929	38	McCulloch.	78	19	—	—	—	97	1.52	—	4	do.	24
1928	48	Perfect Peachblow.	115	12	—	—	—	127	0	—	4	do.	24
1928	59	Prolific.	106	9	—	—	—	115	0	—	4	do.	24
1929	8	do	156	4	—	—	—	160	0	—	4	do.	24
1928	23	Pride of Multnomah.	104	—	—	—	—	104	0	—	4	do.	24
1928	40	Carman No. 1.	207	11	—	—	—	218	0	—	4	do.	24
1928	19	King of the Roses.	107	8	—	—	—	115	0	—	3	do.	24
1928	10	Maggie Murphy.	102	13	—	—	—	115	0	—	3	do.	24

^a Pollen classes as defined by Stout and Clark (18, p. 67).^b South American.^c Groups as defined in text, p. 876.

The chromosomes of three yellow-fleshed varieties (Nos. 302, 336, and 345) were very easily counted. The 12 bivalent chromosomes were frequently seen arranged on the metaphase plate of the first reduction division. (Fig. 1, D, and pl. 1, K.) The chromosomes divide regularly in both reduction divisions of the developing pollen mother cells, and counts could also be made when the chromosomes were on the metaphase plates of the second division. (Fig. 1, E, and pl. 1, O.) It was exceptional to find univalent chromosomes at diakinesis, or unequal distribution of the chromosomes to the four nuclei of the pollen tetrad.

The chromosome behavior of the remaining 37 varieties was frequently so variable that chromosome counts were often made with difficulty. Whenever both univalent and bivalent chromosomes were present it was necessary to distinguish between the two types in order to make counts accurately. Because of the small size and the large number of chromosomes there was danger of making mistakes. Root-tip material from four varieties--McCormick, Green Mountain,

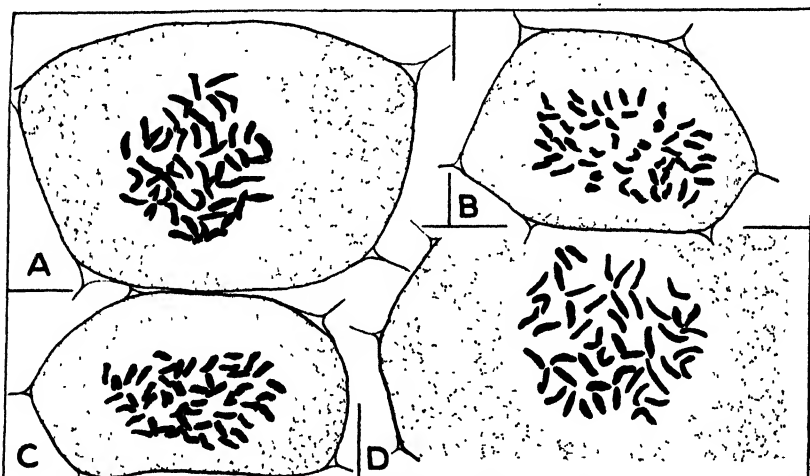


FIGURE 4--Root-tip cells from four potato varieties, each showing 48 somatic chromosomes. A, Early Rose; B, Charles Downing; C, McCormick; D, Green Mountain $\times 2,000$

Early Rose, and Charles Downing--was prepared and somatic counts made. A to D of Figure 4 show representative cells from the four varieties, in which 48 somatic chromosomes are distinguishable.

Pollen mother-cell preparations of all varieties showed chromosome numbers that left little doubt that they should be assigned to the tetraploid group, and the somatic count of four representative varieties supports the conclusion that there is no variation in chromosome number in the commonly cultivated varieties of potatoes.

The meiotic behavior of the chromosomes in developing pollen mother cells in this tetraploid group of potatoes shows nothing of the constancy observed in the chromosome number, and abnormalities are so varied that each variety would require individual examination to picture accurately the varying methods of pollen formation characteristic of it. On the basis of variation in chromosome behavior, the tetraploid varieties have been separated into four groups, as fol-

low, both for convenience and to emphasize the different types of irregularities.

GROUP 1.—Varieties in which there are frequently some unpaired chromosomes among the bivalents, but in which both the first and second reduction divisions usually occur.

GROUP 2.—Varieties in which there are frequently some unpaired chromosomes, with an occasional cell showing no pairing, and in which there are some cells that go through one division only.

GROUP 3.—Varieties in which pairing is variable and in which pollen mother cells rarely go beyond one division and a number of the cells show no pairing of chromosomes.

GROUP 4.—Varieties in which a large percentage of the cells show the somatic chromosome number and in which true meioses are frequently absent. The nucleus degenerates in the prophase or metaphase and the cell divides in an amitotic manner.

The seven varieties—Keeper, Garnet Chili, Never Rot, Prince Albert, two seedlings (Nos. 43225 and 43986, selected because of their fertility), and McCormick—fit best the conditions described for Group 1, although McCormick in 1929 came nearer the conditions described for Group 2.

Each of the foregoing varieties gave suitable material from which chromosome counts could be made in metaphases of both the first and second reduction divisions. Figure 5, D, is a metaphase of the first-reduction division of McCormick, showing 24 bivalent chromosomes. E and F of Figure 5 are metaphases of the second division of Prince Albert and seedling No. 43225, respectively. In E both plates show 24 chromosomes, while F shows 24 chromosomes in one plate and an extruded chromosome in the cytoplasm which had failed to be included in the plate shown in side view.

Although a majority of the pollen mother cells go through their reduction phases normally, unpaired chromosomes are often seen in the early phases of the first reduction division, and if some fail to be included in the two daughter cells there will be more than the usual four cells produced from a single mother cell.

Plate 1, A-I, is a series of photomicrographs that illustrate characteristic phases in chromosome distribution of plants belonging to this most regular group of tetraploid potato varieties. A and B are first-division metaphases of Prince Albert and seedling No. 43752, showing side views of the chromosomes on the plate. It is exceptional to find in either of these varieties univalents scattered on the spindle. C and D are interkineses of Keeper and Prince Albert, respectively, showing for each cell two equal-sized nuclei. E, F, and G are second-division metaphases of Keeper and McCormick—a phase seldom seen except in the seven varieties of this group. H shows second-division anaphases of Keeper, and I 4-celled tetrads of seedling No. 43752. It is difficult to photograph a tetrad with all four cells showing,

EXPLANATORY LEGEND FOR PLATE 2

A.—Second-division metaphases in Peachblow.

B and C.—Two-cell, 3-cell, and 4-cell pollen groups in Peachblow.

D.—Metaphases in Russet Rural.

E.—Metaphases in Blue Victor showing the regular plates in cells when all chromosomes are unpaired.

F.—Telophases in Russet Rural.

G and H.—Telophases in Peerless (Pearl)

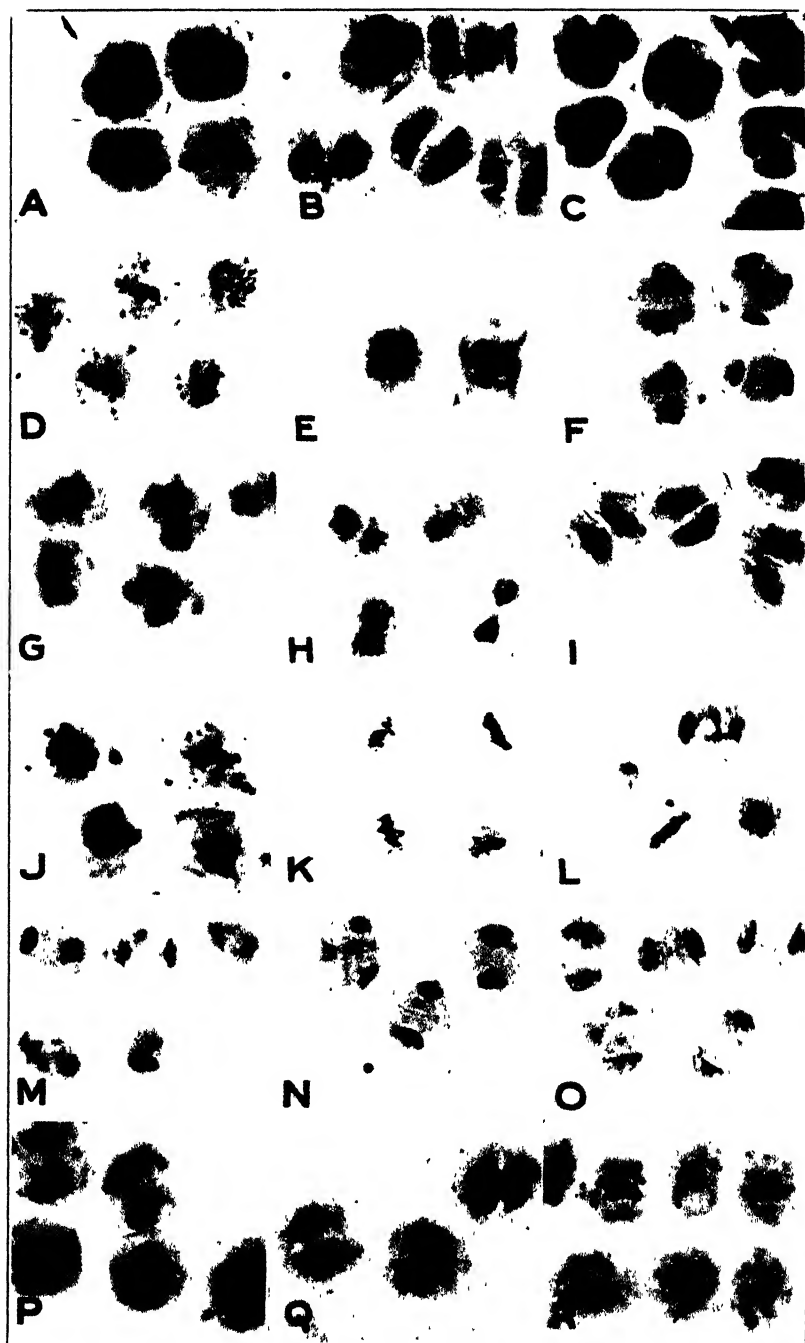
I.—Two-cell pollen groups in Dakota Red.

J, K, and L.—Metaphases in Charles Downing, many of which show no pairing of chromosomes.

M, N, O, and P.—Telophases in Prolific, Charles Downing (N and O), and Cowhorn, respectively.

Q.—Two-cell pollen groups in Cowhorn.

R.—First-division metaphases in *Solanum fendleri* × *S. chacoense*.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

as the fourth cell is usually in a different plane from that of the other three.

Four varieties—Green Mountain, Irish Cobbler, White Albino, and Peachblow—and also McCormick collected in 1929, fit the behavior described for Group 2.

From 25 to 75 per cent of the pollen mother cells of the above-listed varieties pass through both reduction divisions in a fairly regular manner, except for the presence of univalent chromosomes, which are distributed at random or extruded.

A and B of Figure 5 are first-division metaphases of Green Mountain and a variant of Green Mountain known as Giant Hill, respectively. The haploid number, 24, is shown, but it is exceptional to find a cell with all chromosomes paired. These cells, in which all chromosomes are paired, are pictured because they show best the

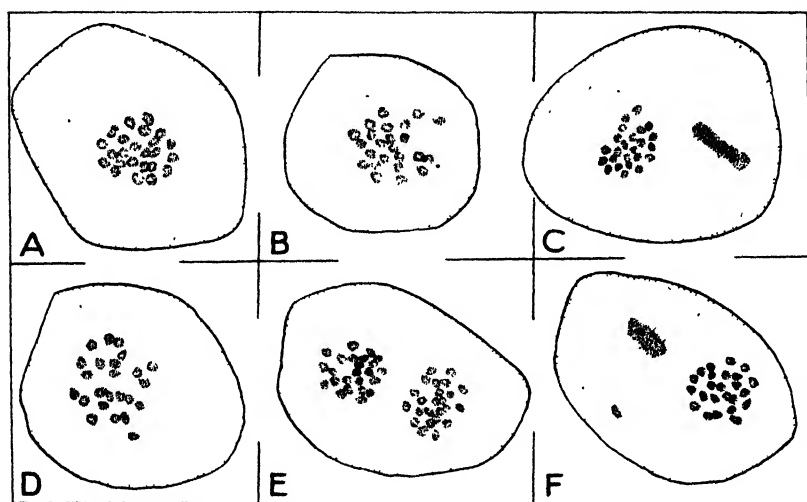


FIGURE 5. Pollen mother cells from potato varieties. A, B, and D, First-division metaphases of Green Mountain, Green Mountain Giant Hill, and McCormick, respectively. C, E, and F, Second-division metaphases in Green Mountain Giant Hill, Prince Albert, and seedling No. 43225, respectively. $\times 2,000$

true chromosome number for these varieties. A regular second-division metaphase of Green Mountain Giant Hill is illustrated in Figure 5, C.

The chromosome behavior that separates these varieties from those of Group 1 is the occasional presence of a cell showing no paired chromosomes and the frequent appearance of cells in which there is no second division, so that many pollen mother cells produce only two pollen grains. Occasionally, however, chromosomes are distributed into three groups, or even more, giving consequently three or more pollen grains from cells that never make more than one division.

Plate 2, A–C, shows three photomicrographs of Peachblow. A is a second-division metaphase—a phase that is found much less frequently than in varieties of Group 1. B and C show pollen groups of this variety. The number of cells from a mother cell is variable, and these photographs show 2-cell, 3-cell, and 4-cell pollen groups that have been produced by sister cells.

Group 3 includes the following 16 varieties, and in all of the the number of cells making both first and second divisions is less than 10 per cent and in most varieties almost zero:

American Giant.
Beauty of Hebron.
Blue Victor.
Dakota Red.
Early Manistee.
Early Ohio.
Early Rose.
Early Sunrise, Buist's.

Jersey Red Skin.
Noroton Beauty.
Peerless.
Peoples.
Queen of the Valley.
Russet Rural.
Scotch Rose.
Triumph.

Almost any preparation in which pollen mother cells of these varieties are found will contain some cells showing no chromosome pairing. These cells often afforded an opportunity to obtain chromosome counts in varieties in which regular pairing was seldom seen. In cells having both univalent and bivalent chromosomes it is difficult to count the chromosomes accurately, and the studies were made easier by the presence of cells showing no paired chromosomes. The number of cells containing the full somatic number varied in different varieties.

B and C of Figure 6 show two cells from a preparation of Beauty of Hebron, one having 48 small univalent chromosomes, the other 24

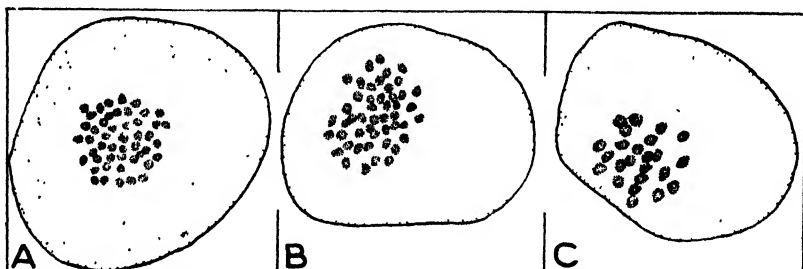


FIGURE 6. —Pollen mother cells from potato varieties. A, Metaphase of Prolife showing no chromosome pairing. B and C, First-division metaphases of Beauty of Hebron showing 48 univalent chromosomes and 24 bivalent chromosomes, respectively. $\times 2,000$

larger bivalent chromosomes. Two similar cells of Peoples are shown in Figure 7, A and B. E and F of Figure 7 are cells of Blue Victor and Triumph, respectively, which have been crushed slightly to separate the chromosomes. They serve to compare the size of univalents and bivalents. In E there are 22 univalents that either failed to pair or have divided after pairing and 13 paired chromosomes, while in F there are 23 paired and 2 univalent chromosomes.

Cell division often follows immediately after a single division of the chromosome, and consequently a single pollen mother cell produces only two pollen grains, except when the chromosomes are distributed into more than two groups or when no division of the cell takes place, giving, consequently, from a single mother cell, one or three or more pollen grains.

In Plate 2, D-I, is presented a series of photomicrographs illustrating the characteristic procedure in pollen formation of varieties of Group 3. D shows metaphases of Russet Rural with paired chromosomes on the plate and univalents scattered on the spindle. E shows two cells of Blue Victor in which there was no chromosome pairing; the 48 univalent chromosomes form a regular plate very similar to

that seen in a somatic cell. F, G, and H show cells of Russet Rural and Peerless before cell division has taken place. Two or three nuclei produced from a single irregular nuclear division may be seen. I is a typical photograph, showing 2-celled pollen groups of Dakota Red that have resulted from a single division of the pollen mother cell.

The fourth group includes Charles Downing, Prolific, McCulloch, Cowhorn, Perfect Peachblow, Russet Burbank, and Scotch Rose. Scotch Rose stands between Groups 3 and 4, showing characteristics sometimes of the one and sometimes of the other. This variety, therefore, is included in both groups.

The outstanding characteristic of a group of cells from a preparation of any of the foregoing varieties is the prevalence of cells having 48 unpaired chromosomes. One would be led to class these varieties as octoploid except for the presence of cells showing paired chromosomes, mixed with others showing no chromosome pairing. The

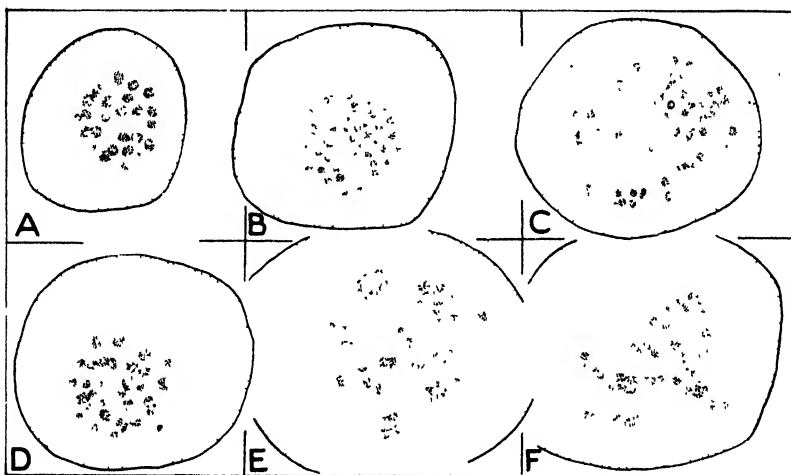


FIGURE 7 - Pollen mother cells from potato varieties. A, Metaphase from Peoples showing 22 bivalent and 4 univalent chromosomes, B, metaphase from Peoples showing 48 univalent chromosomes, C, late metaphase from Charles Downing, D, metaphase from McCulloch showing 16 univalent and 16 bivalent chromosomes; E, late metaphase from Blue Victor showing univalent and bivalent chromosomes, F, metaphase from Triumph showing divided and dividing chromosomes. $\times 2,000$

chromosomes in cells containing 48 chromosomes agree in size with the univalents in cells in which there is some pairing. The writers do not hesitate to place these 48-chromosome varieties in the tetraploid group, especially since one representative—the Charles Downing—was found to have 48 chromosomes in the root-tip cells.

Very little can be said about the behavior of the chromosomes during the meiotic phases. Many cells do not show clearly formed chromosomes, and others that do show chromosomes fail to go beyond the metaphase. The chromosomes degenerate early, and the nucleus divides amitotically at the time the cell is undergoing division.

Figure 6, A, shows a metaphase of Prolific with 48 chromosomes on the plate. C and D of Figure 7 are also metaphases of Charles Downing and McCulloch, respectively, which have both paired and univalent chromosomes. These three figures serve only to show the number and size of chromosomes. The behavior of most cells was so

variable that no single drawing could be given as typical. Many pollen mother cells seemed to be in the early stages of degeneration, and only in a very small percentage of the cells was there a true meiotic division; there were no cases where cells went into a second-reduction division, so that the least abnormal pollen mother cells made only two pollen grains.

Plate 2, J-Q, is a series of photomicrographs of some of the more regular-behaving cells from representatives of Group 4. J-L are metaphases in Charles Downing showing scattered univalents in cells in which there was some chromosome pairing and more regular plates in cells in which there was no chromosome pairing. M-P show cells of Prolific, Charles Downing, and Cowhorn just before cell division starts. Most of the cells have two nuclei; a few have three unequal-sized nuclei resulting from a single nuclear division. Q shows three pollen groups of two cells each of Cowhorn. Such 2-celled groups are typical of varieties of this group whenever the pollen mother cell has divided. Groups of three cells occasionally arise from cells in which the nucleus has divided into three nuclei, but groups with more than three cells occur very infrequently.

Four other varieties—Maggie Murphy, Carman No. 1, King of the Roses, and Burbank—were included in the 1928 studies. Detailed observations are lacking, but a general study was made which was sufficient to show that they should not be included in either of the more regular groups, 1 or 2.

POLLEN TETRAIDS

Much has been said in the preceding section of the production of pollen grains from a single pollen mother cell. Normally it is expected to find a group of four cells produced from a single pollen mother cell. To this group has been given the name "pollen tetrad." If, however, only two cells are produced, the name tetrad hardly can be applied even if modified by the term "irregular."

A study of the number of cells in a group produced by a single pollen mother cell gives a fairly reliable picture of the prevalence of irregularities in the reduction division. If there is a predominance of 4-celled groups, the variety is regular; if there is a predominance of other than 4-celled groups, the variety is irregular.

In Table 2 are shown for each variety the prevalent type of reduction division, the number of pollen groups with four cells, the number with more or less than four, and the character of the mature pollen. The figures for the character of the mature pollen were taken from Stout and Clark (18) or calculated from observations made by the junior writer.

From Table 2 it can be seen that, with the exception of Prince Albert, those forms classed in Group 1 on the basis of chromosome behavior during pollen formation produce from 75 to 100 per cent 4-cell pollen tetrads and are placed in either class 1 or 2 of the four mature pollen classes described by Stout and Clark (18). It is difficult to explain why the mature pollen is poorer in Prince Albert than in others of this group.

Varieties of Group 2 are seen to give between 25 and 75 per cent 4-cell pollen tetrads and have been put in either class 2 or 3 according to the character of their mature pollen. In Group 3 there is very

little production of 4-cell tetrads, and all varieties belong to either class 3 or 4 according to the character of their mature pollen. Group 4 rarely produces a 4-cell tetrad, and all are in class 4 in the character of their mature pollen.

It seems that in a general way one may attribute poor pollen production to some abnormality in the chromosomes at the time of pollen formation. There are few exceptions, and the agreement seems sufficiently close to justify the conclusion that a study of the pollen mother cell development will show the character of the mature pollen of a variety or vice versa.

EFFECT OF ENVIRONMENT

It has often been thought that a variety is more fruitful in one year than in another, and a study of the pollen tetrads in both 1928 and 1929 was undertaken to determine whether the differences in environment in the two years were sufficient to affect the pollen production of a variety.

Twelve varieties of Groups 3 and 4 were studied in both 1928 and 1929, but the production of 4-cell tetrads was under 5 per cent in the 24 counts made. No significant difference could be detected in the counts of any one variety made in two different years. It seems reasonable to assume, therefore, that varieties in these two classes are uniformly irregular in their chromosome behavior and are poor pollen producers under normal but variable growing conditions.

The two varieties of Group 2 in which pollen tetrads were counted in both 1928 and 1929 proved more interesting. Peachblow, for example, in 1928 had over 90 per cent 4-cell tetrads. On the basis of these counts it should have been put in Group 1, but counts from different buds made in 1929 gave an average of about 47 per cent. In Green Mountain the counts made in 1928 show about 43 per cent, while the counts in 1929 average about 5 per cent, varying from 0 to 11 per cent.

In Group 1 two varieties were studied in both 1928 and 1929. Prince Albert, with an 80 per cent production of 4-cell tetrads, was constant over the two years, while McCormick showed 75 per cent 4-cell tetrads in 1928 and only 57 per cent in 1929.

It is apparent from tetrad counts given in Table 2 that the production of 4-cell tetrads varies in varieties that are intermediate between the more fertile and the more sterile classes.

Three of these intermediate varieties—McCormick, Peachblow, and Green Mountain—showed a variable production of 4-cell tetrads in 1928, when samples were taken from buds growing on adjacent plants of the same variety. The counts are not in sufficiently close agreement to ascribe the variability to random sampling alone. Some factor or factors have affected the chromosome behavior during the meiotic phases of pollen formation. The environment in which a plant grows is often sufficiently variable to cause noticeable differences in growth of adjacent plants. The position of a bud in a flower cluster may be favorable or unfavorable, causing considerable variation in the development of individual buds. One is tempted to ascribe the variability in the production of normal pollen of different buds of a progeny to varying environmental conditions.

The growth conditions of two different seasons are not likely to be identical. A study of the production of normal tetrads in the

above-mentioned three varieties was made in 1929, and a variation similar to that of 1928 was found when samples from different buds of the same progeny were compared, but in addition it was found that the production of 4-cell tetrads in all these varieties was lower than in 1928. The differences were too great to be ascribed to random sampling and suggested that growth conditions in 1929 must have been more unfavorable for normal chromosome behavior at the time of pollen formation than in the preceding year.

An examination of weather charts, kindly lent by the superintendent of the Aroostook Farm, showed a difference in the rainfall of the two years, during the week preceding the collection of material, that may have been sufficient to change the growth conditions of potatoes. In 1928 there was ample rain--1.29 inches for the week preceding the collection from McCormick and 1.44 inches for the week preceding the collection from Peachblow and Green Mountain. In 1929, for the same period, there was only 0.8 inch preceding the collection from Green Mountain and no rain at all preceding the collection from Peachblow and McCormick.

That deficiency in soil moisture might be responsible for the more irregular meiotic behavior of 1929 is only suggested. It is the only climatic factor showing a wide difference between the two years, but an extensive study would be required before it could be stated definitely to what extent specific environmental factors modify chromosome behavior during gametic formation.

It seems apparent that in different varieties of potatoes the chromosome complement does not show the harmony observed in wild species. This lack of harmony is shown in varying degree in the varieties listed in Table 2. So pronounced is it in a large percentage of the varieties that there is little or no normal pollen mother-cell development. In a few varieties the chromosomes seem to be more in harmony, and an appreciable number of normal tetrads are produced. This harmony, however, seems easily disturbed, and a comparison of material from different buds collected either in the same year or in different years shows a variability too great to be ascribed to random sampling and suggests that variable growth conditions have affected the chromosome behavior. In a very few varieties the chromosome complement seems to be in harmony and the meiotic behavior is almost as regular as that found in material from wild species.

DISCUSSION

It is apparent from this study that polyploidy is prevalent in the potato and some of its near relatives. Forms have been described with 12, 18, 24, and 36 as their haploid chromosome number. A survey of cytological literature shows that it is unusual to find a species represented by both a diploid and a tetraploid form. The diploid representatives of *Solanum tuberosum* include a small group known as the yellow-fleshed varieties of South America. In them it was found that the haploid chromosome number is 12, while the other 37 cultivated varieties examined in this study all have 24 as their haploid chromosome number. Fukuda (5) made a similar study of the chromosomes in 40 varieties of potato and states that the haploid chromosome number for each of them is 24.

Earlier investigators, i. e., Němec (14), Martins & Mano (12), Müller (13), and Lutman (11) describe varieties of *Solanum tuberosum* with chromosome numbers of about 36. More recent studies by Levitsky and Benetskaja (10) and the author referred to in the preceding paragraph seem to make these lower counts unlikely. Levitsky finds in addition to the normal 48 somatic chromosomes that there is a tendency for chromosomes to fragment, which gives the impression that the chromosome number is above 48 in some cells.

The universal presence of the tetraploid chromosome number in our North American cultivated varieties of potatoes precludes the use of chromosome counts as a means of identifying potato varieties. This double chromosome number, however, may be significant from an evolutionary standpoint. It is now generally believed that a form with the basic chromosome number is not as far removed from the ancestral types as polyploid forms. If we consider this to be the case in *Solanum tuberosum*, we must place the yellow-fleshed varieties near the ancestral type. The regular behavior of the chromosomes during the reduction divisions certainly indicates for them a much more stable ancestry than that suggested by the nonharmonious behavior of chromosomes in the tetraploid varieties.

The presence of irregular meioses in potatoes during pollen formation was referred to by Young (23), who says that occasionally the homotypic division is omitted. Fukuda (5) considers the chief cause of pollen sterility to be due to the abnormal meiotic behavior of the developing pollen mother cells. Stow (19), who saw from the work of the two preceding authors the possibility that external conditions might be the cause of irregular pollen mother-cell development, subjected developing buds to extremes of heat and cold. He found that varieties normally behaving regularly will behave irregularly when the temperature is appreciably increased or decreased. Stow's conclusions, however, are hardly applicable to varieties that behave irregularly under the usual growth conditions.

The pollen development in tetraploid potatoes seems to the writers to be disturbed by inherent factors that make the production of normal tetrads exceptional in a large percentage of the varieties studied by the writers. A detailed examination of the types of pollen tetrads produced by three of the more fertile varieties suggests that, in addition to their inherent factors, environment may modify the chromosome behavior during meioses, which in turn determine the character of the pollen tetrad and eventually the amount of viable pollen produced by a variety. A comparison of material collected in 1928 and 1929 showed that in both years the variation in buds from different plants was large, which may be explained by differences in growth conditions of the plant or in the position of the buds on an individual plant. A comparison of the results for the two years indicates that the conditions in 1928 were more favorable for pollen formation than in 1929.

Although environment may modify somewhat the chromosome behavior during the reduction divisions of the developing pollen mother cells, the writers agree with Fukuda (5) that the lack of harmony of the chromosome complement of tetraploid varieties of potatoes is the major cause for the failure to produce, in potatoes, normal 4-cell pollen tetrads.

The writers, for convenience and because the irregularities in pollen mother-cell development show characteristic differences, have divided their material into four groups based on the predominance of some characteristic irregularity.

There is first the variable amount of chromosome pairing. Failure of some of the 48 chromosomes to pair could be found in all cultivated varieties; in some, however, it was much more prevalent than in others. Varieties in which the only noticeable irregularity in chromosome behavior during meiosis is the failure of some chromosomes to pair, produce a fair amount of viable pollen and are considered our most fruitful varieties.

Combined with the irregularity in the pairing of the chromosomes there may be one or all of the three following unusual abnormalities: (1) Absence of a second reduction division; (2) absence of any tendency for chromosomes to pair; (3) early degeneration of chromosomes, followed by an amitotic division.

Fukuda (5) described very similar irregularities in his discussion of the chromosome behavior of potato varieties, although he has grouped the irregularities differently from the writers. Irregular phenomena are so varied and numerous in many of the pollen mother-cell preparations that it would require a long study to interpret and describe them all. The writers have described only the more prevalent irregularities that seemed characteristic of any particular variety or that facilitated obtaining an accurate chromosome determination.

The chromosome behavior of tetraploid potato varieties is frequently sufficiently characteristic to enable one to distinguish by a cytological study varieties in the more fertile classes from those of the sterile class, but this character would be of little service in distinguishing varieties of the same group.

What the writers have found concerning the production of viable pollen only confirms what potato breeders have known for some time. Only a few varieties are useful as pollen parents in potato crosses. The writers have, however, traced one step farther back the cause of nonfruitfulness in potatoes. It is due to the absence of viable pollen, which in turn is due to an abnormal chromosome behavior at the time of pollen formation.

Many of the recent studies of polyploid species suggest explanations for the abnormal chromosome behavior so prevalent in tetraploid potato varieties. But perhaps the recent study of Karpechenko (8) best fits the conditions described in this study. In an investigation of the chromosome number and behavior of *Raphanus sativus* L. and *Brassica oleracea* L. he found that both species have the basic chromosome number 9 and that their chromosome behavior is regular. F_1 hybrids of these two species had the same chromosome number as the parents, but the chromosome complement was so inharmonious that no chromosome pairing occurred and pollen development was very irregular, giving gametes with varying chromosome numbers. F_2 plants were found to have from 27 to 78 somatic chromosomes, and the meiotic behavior during pollen formation was varied and strikingly abnormal. Karpechenko's material illustrates well the origin of polyploid forms from hybrids as due to the characteristic irregular chromosome behavior of a hybrid in which the chromosome complement is made up of two distinctly different sets.

In the potato the conditions are reversed. One presumes that nature has been the hybridizer and that tetraploid potatoes represent the outcome. Karpechenko (8) points out that if his plants were to propagate apogametically polyploid forms would be established. In potatoes a form becomes established through asexual reproduction, so that a perfectly sterile form may be as long-lived as a more fertile one.

All of our tetraploid potato varieties have a chromosome behavior that suggests a mixed ancestry. If one were to construct a chart showing the possible descent of our cultivated potatoes, it would begin with two or more wild *Solanum* species having the basic chromosome number 12. From the hybrid progeny of these species have arisen, just as from Karpechenko's hybrid, new forms with double the number of chromosomes, whose hybrid character has been preserved through long periods by asexual reproduction.

The hypothesis of the hybrid origin of polyploid cultivated potatoes is strengthened by the existence of a group of closely related polyploid wild forms. Three of these—*Solanum commersonii*, *S. cardiophyllum* f. *coyoacanum*, and a hybrid resembling *S. maglia* referred to by Clark (2)—have a chromosome behavior that strongly suggests that they are natural hybrids and not regular-behaving species. The number and behavior of the chromosomes seem to show that considerable natural crossing occurs among wild species in their native habitat.

Irregular behavior has been duplicated in an artificially produced hybrid of *Solanum fendleri* \times *S. chacoense* in which a 24-chromosome set or two 12-chromosome sets are combined with a 12-chromosome set. The irregular meiotic behavior in the developing pollen mother cell is typical of a hybrid produced when plants with different chromosome numbers are crossed.

The heterozygous condition of practically all of our potato varieties for many of their most prominent characters has also been regarded by some as an indication of a hybrid origin. On the other hand it is pointed out by Fukuda (5) and by Wittmack (22) that the shape of the flower parts, which is an important character in the differentiation of species, has not changed since the time of the earliest descriptions of the potato, over three centuries ago. The constancy of the floral characters is also confirmed by the observations of the junior writer, which are based on the progenies of a large number of crosses among many varieties. This, however, is not a serious obstacle to the theory of a multiple origin, as it is possible that the original progenitors of the present species may have possessed the same type of calyx and corolla and yet have differed in many other characters. If more than one type of calyx and corolla were involved in the early ancestry, it would be possible for the dominant type, especially if linked with desirable economic characters which would insure its preservation, to be carried along for several generations by the usual methods of potato breeding, which consist for the most part in selecting and propagating vegetatively the first or occasionally the second generation of crosses between highly heterozygous parents. So far as the writers are aware, no taxonomic studies have been made of pure lines of potatoes which have been carried through several inbred generations. While in the yellow-fleshed varieties included in these studies the calyx and corolla are of the same general type as those of the

white-fleshed varieties, the differences in the cytological behavior, the number of chromosomes, and other characteristics suggest the probability that these two groups are not of the same immediate ancestry.

Cultivated potatoes have been subjected to a long period of asexual reproduction. This allows the preservation of a herbaceous form that would immediately be lost if it depended on seed reproduction. Has asexual reproduction been the cause of the degeneration of the gametic cells? One of the first plants in which abnormal meioses were described was *Heimerocallis fulva* (Juel (6)), a plant that has long been subjected to asexual reproduction, but it is still in doubt whether asexual reproduction has been the cause of abnormalities, since here also they may have been due to the hybrid ancestry of the plant. No authentic case seems to be on record of a regular-behaving species whose fruitfulness has been affected by subjection to long asexual reproduction. On the other hand, very many of our cultivated plants that are propagated asexually often show considerable tendency to be nonfruitful; but they are plants in which hybridization has probably occurred, and the origin of sterility well may be attributed to incompatible chromosome combinations which have been perpetuated by continued asexual propagation.

The investigations of Krantz and Hutchins (9) in potato breeding indicate at the present time that a potato variety becomes more uniform after three or four generations of self-fertilization. This effect of inbreeding may be taken as a further demonstration that the domesticated potato has retained the characteristic irregularities of inharmonious chromosome complements simply because it has been propagated asexually. Sexual generations provide an opportunity for gametic selection to eliminate the unbalanced chromosome combinations typical of hybrids between species, and thus result in the production of stable forms.

The conclusions of the writers are at variance with those of Fukuda (5), who considers the domesticated potato to have arisen from a single species. It seems more probable that the cultivated potato has a very mixed ancestry, as held by Juzepchuk and Bukasov (7), and that the ancestors of the 24-chromosome varieties of *Solanum tuberosum* are to be found not in a single wild species but in two or more.³

SUMMARY

This paper presents a study of the number and meiotic behavior of chromosomes in tuber-bearing forms of *Solanum*.

Ten wild species of potato were found to have 12, 18, 24, or 36 as their haploid chromosome number. Two species, *Solanum comersonii* and *S. cardiophyllum* f. *coyoacanum*, with 18 as their haploid chromosome number, have meiotic irregularities in their pollen

³ After the manuscript of the present paper was completed, two papers by Rybin (15, 16) were received that are in agreement with several of the conclusions drawn from a study of the material available to the writers. Rybin is of the opinion that all the European and North American commercial varieties of potato probably have 48 as their somatic chromosome number. He finds forms of wild potatoes with 24, 36, 48, 60, and 72 somatic chromosomes. The triploid forms were found to have irregular reduction divisions similar to those described by the writers, and the pentaploid forms were known either to be hybrids or to have characters that suggested a hybrid ancestry. He finds among the local cultivated potato varieties of Central America and South America forms with 24, 36, and 48 chromosomes. His study of the morphological and cytological characters of the triploid forms has led him to conclude that they are hybrids which have either originated from a cross of cultivated species having 24 and 48 chromosomes, respectively, or descended from 36-chromosome wild forms. In his cytological data he gives strong support to the hypothesis that cultivated potatoes have a polyphyletic origin.

mother cells similar to those found in a known F_1 hybrid, *S. fendleri* \times *S. chacoense*, suggesting that these two wild species are natural hybrids.

Three cultivated varieties of *Solanum tuberosum* grown in South America have 12 as their haploid chromosome number.

Thirty-seven cultivated varieties of *Solanum tuberosum* grown in the United States have 24 as their haploid chromosome number.

The meiotic behavior of the 24-chromosome *Solanum tuberosum* varies from regular in a few to extremely irregular in many of the varieties.

Only the few varieties with a regular chromosome behavior produce an appreciable amount of pollen; varieties with an irregular chromosome behavior produce practically no pollen. Unfruitfulness in potatoes would therefore seem to be due to abnormal chromosome behavior at the time of pollen formation.

The chromosome behavior of a few selected individuals seemed to be affected to some degree by environmental changes.

The number and behavior of the chromosomes in our cultivated potato varieties suggest that they have a mixed ancestry and that the ancestors are to be found not in a single wild species but in two or more.

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